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GLUTAMATES

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SUMMARY

Description and Specifications

Glutamic acid ($C_5H_9NO_4$) is a white, practically odorless, free-flowing crystalline powder of molecular weight 147.13. It is slightly soluble in water, forming acid solutions, and is practically insoluble in ether, acetone, or cold glacial acetic acid. It decomposes at 247-249°C. The Food Chemicals Codex (1486) specifies that food grade glutamates should contain no more than 0.0003% arsenic nor more than 0.001% lead.

Monoammonium glutamate (MAG) ($C_5H_{12}N_2O_4 \cdot H_2O$) is a white, practically odorless, free-flowing crystalline powder of molecular weight 182.18. MAG is freely soluble in water, but is practically insoluble in most organic solvents.

Monopotassium glutamate (MPG) ($C_5H_8KNO_4 \cdot H_2O$) is a white, practically odorless, free-flowing, hygroscopic crystalline powder of molecular weight 203.24.

Monosodium glutamate (MSG) ($C_5H_8NaNO_4 \cdot H_2O$) is a white or almost white, nonhygroscopic, crystalline powder of molecular weight 187.13. MSG is very soluble in water but is only sparingly soluble in alcohol.

Glutamic acid hydrochloride ($C_5H_8NO_4 \cdot HCl$) is a white, crystalline powder of molecular weight 183.59. It

decomposes at 214°C and is nearly insoluble in alcohol and ether; 1 g dissolves in about 3 ml of water.

Acute Toxicity

Monosodium Glutamate

The following median lethal doses (LD_{50} in mg/kg body weight) have been established for oral administration: mouse, 1920, rat 1660 (5804); intraperitoneal (i.p.) administration: rat, 3600 (3829); subcutaneous (s.c.) administration: chickens, 3000-4000 (1155). The LD_{100} for s.c. administration to the chicken has been found to be 5000 mg/kg BW (1155).

Monoammonium Glutamate

The LD_{50} for i.p. administration to the rat has been found to be 1000 mg/kg BW (3829).

Toxic Effects: Retinal Lesions

The discovery that glutamates could be neurotoxic has been credited by most of the authors cited below to Lucas and Newhouse. In 1957 they reported (4487) that single s.c. doses of MSG 4 g/kg and up produced retinal lesions in neonatal mice within a few hours of treatment. Others, largely following their methods, found similar lesions after single or daily doses of MSG (summarized in Table 13 on p. 49), the smallest being 0.5 g/kg i.p. for 16 days in adult rabbits (2770). These authors included Potts et al. (5878), Cohen (1432), Olney (5481), Hansson (2804),

and Kobayashi (3862). Another author (1091) later commented that MSG was declared Generally Recognized As Safe (GRAS) the year after the Lucas and Newhouse report.

Electronmicroscopy indicated that primary damage was confined to the inner cell layers, leaving the photoreceptors apparently intact (1432). Damage to supporting blood capillaries and related appearance of glial cells were noted (2805), and indicators of damage were found in the electroretinogram (ERG) (5878). Most of the studies were descriptive, but one group of workers found evidence to suggest that the direct cause of damage was endproduct-repression of the enzyme glutaminase I (2236). However, this theory was disputed (5481).

Toxic Effects: Brain Damage, Neuroendocrine Disturbances, and Nephro-toxicity

Rodents. In 1969 Olney was prompted to examine the hypothalamus by observing that some mice given MSG in a retinal study later became fat (5488). He found lesions of the arcuate nucleus but not of the ventromedial nucleus. [In 1973 Gold reported that obesity did not result from ventromedial damage but only from damage to the adjacent ventral noradrenergic bundle or its terminals (6315).] The smallest effective doses were single doses of MSG 0.5 g/kg s.c. (5488); higher doses daily for 10 days after birth produced, 9 months later, a record of smaller food intakes, smaller skeletal growth, heavier mice due to

adiposity, smaller anterior pituitaries, and sterility in females but not in males.

Further reports from the same laboratory (5492, 5486) indicated that the smallest effective oral dose of MSG for arcuate lesions in neonatal mice was 1 g/kg for 100%, and 0.5 g/kg for 50%; no lesions were seen with 0.25 g/kg or less. Of 24 compounds related structurally to MSG, glutamic, aspartic, and cysteic acids were reported as neurotoxic, and the authors concluded that the significant association was with neuroexcitatory properties. Van Gelder (7648) came to a similar conclusion after feeding L-glutamic acid, glycine, or γ-aminobutyric acid (GABA) to mice.

Brain lesions have also been attributed to depressed uptake of glucose (1547), but this finding has been disputed (6313).

Other workers then reported similar findings, at doses that are summarized in Table 19 on p. 64 and in Table 21 on p. 70. However, not all reports were confirmatory. Arees and Myers (0307) reported at first that they found hypothalamic changes that did not involve neurons, but in a later report (0305) they saw neural lesions in subiculum and lateral geniculate nuclei and concluded that damage was worse than they had suspected. Oser et al. (5530) found no changes that they considered significant in mice, rats, or dogs that were given MSG 1 g/kg orally or s.c. and killed 24 hours later. Adamo and Ratner (0050) gave rats MSG 4 g/kg s.c., killed some after three hours and

others after two to three months, and found no preoptic or arcuate damage and no reproductive effects in females or males.

To resolve these discrepancies Burde et al. (1053) performed a blind study in mice and rats, using Olney's published methodology and employing independent examiners for tissues. The results confirmed Olney's work (5488, 5492), and the authors (1053) commented that on close scrutiny the only data that appeared to conflict with Olney's were those of Adamo and Ratner (above).

Further reports on rodents given MSG disclosed more extensive brain damage in areas adjacent to cerebrospinal fluid (CSF) (4327), increased lysosome populations in neurons, glia, and ependymal cells of the arcuate nucleus (0032), transplacental induction of brain lesions in fetuses (5130), and fatty livers with delayed puberty (4747).

Obesity has been attributed to decreased hormone outputs from smaller hypothalami and pituitaries (6052); others (3859) have reported obesity despite lower adipocyte counts after MSG, together with altered lipid-retention responses of adipocytes to insulin and epinephrine.

Convulsions have been reported in rodents after MSG (i.p. or intragastric) 2-4 mg/kg or MSA i.p. 4 g/kg, but not after glycine or NaCl (5143); in another study pyridoxine appeared to be involved, upsetting the balance of glutamate and GABA metabolism (0746). A study of the convulsant effects of a number of amino acids given i.p. to juvenile

rats concluded by supporting Olney's thesis of a relationship with neuroexcitatory properties, but no effects were produced in adult rats (3436). In addition, two reports were found of behavioral deficits in rats given MSG orally (7915, 5884). In a behavioral study on mature gerbils, the authors concluded that adult rodents were protected from MSG neuropathology by the blood brain barrier (0581).

Chicks. Brain lesions similar to those of rodents but visible macroscopically were induced by MSG 1 g/kg s.c. (4025). Behaviors and ERG indicators of tectal evoked response to light were depressed or blocked by MSG 4 g/kg i.p., but the authors cautioned against extrapolation to the human (6643).

Severe to lethal avian gout appeared in chicks given drinking-water with 1.3-2.6% MSG; different and milder renal pathology was seen in others offered saline, and none was seen in others offered MSG or plain water; the authors concluded that the glutamate as well as the NaCl had contributed to rapid over-elaboration of uric acid (6714).

However, when MSG 1.0 g/kg or less was given daily s.c., or chicks were fed a diet with 0.5-10% MSG, intakes, growth, and mortality were unaffected (1155).

Primates. In 1969 Olney and Sharpe (5490) reported hypothalamic damage to a premature infant rhesus monkey given MSG 2.7 g/kg s.c. and killed three hours later

without having shown behavioral signs. In a reply to immediate criticisms the authors reported that they had also found brain damage in all mice given oral MSG 1 g/kg and in half of some mice given 0.5 g/kg, that the effects of MSG and aspartic acid were additive, and that when a number of amino acids and sodium salts were tested orally and s.c. in mice, only MSG, L-cysteine, sodium L-aspartate, and sodium DL-α-aminoadipate had produced brain or retinal lesions (5491). The MSG had also produced 10-fold rises of blood glutamate in mice (5491).

This was followed by two reports (0032, 6095) of monkeys given MSG 1-4 g/kg without apparent effects on the hypothalamus, and by a detailed report from Olney et al. (5485) on eight more monkeys in which lesions were produced and, in one case, convulsions. Olney et al. (5485) disputed the two negative reports (0032, 6095) on technical grounds, one of which was the emetic effect of MSG. They also estimated the blood glutamate threshold for primate lesions as 20 mg/100 ml, stated that brain damage had been observed at lower doses than had behavior signs, and added that glutamate was a natural metabolite; therefore, they concluded that the total intake of glutamate (in humans) ought to be considered rather than only MSG (5485).

Humans. Randolph and Williams (5997) reported in 1950 that individuals allergic to beets were also allergic to MSG derived from beets by fermentation, and commented that food labels did not disclose this hazard.

Chinese Restaurant Syndrome (CRS) was first reported in 1968 by Kwok (4197), who described symptoms beginning 15-20 minutes after ingestion and lasting about two hours without after-effects. Many reports followed. Ambos (0196) found that females were more susceptible than males. Schaumberg and Byck (6471) described the symptoms under three categories (burning sensation, facial pressure, and chest pain), with an oral threshold for minimum symptoms of 1.5-12 g MSG and a time lag of 15-25 minutes. The i.v. threshold was 25-125 mg with a time lag of 17-20 seconds. Using a cuff they (6471) determined that the "burning" was peripheral. In no subject did they fail to produce symptoms at some level of dosage; in three families with more than one highly susceptible member they failed to find any known genetic pattern. They concluded that contaminants of MSG could be ruled out, and that MSG itself could produce undesirable effects at dose levels widely used (6471).

A negative study was then reported (5084) by authors who criticized Schaumberg and Byck (6471) for methods and criteria, but who themselves were criticized on similar grounds (2993). However, another negative study was reported by Bazzano et al. (0581) in which a cholesterol and β-lipoprotein lowering effect of MSG was also observed.

In 1971 Ghadimi et al. (2474) produced CRS in all of 14 fasted volunteers using MSG 150 mg/kg in 150 ml water and, from biochemical analyses, concluded that the

symptomatology was consistent with neuroexcitation from transient rapid over-elaboration of acetylcholine.

In 1972 Kenney and Tidball (3676) reclassified CRS symptoms as not dose-dependent, highly dose-dependent, or dose-dependent above an appearance-threshold. They suggested that the glutamate in MSG was not physiologically equivalent to the glutamate ingested in dietary protein. Levey et al. (4634) studied the effects of several amino acids given i.v. and concluded that emetic effects were parallel to the free glutamate content of the dose, that toxic effects were related to subsequent serum glutamate levels, and that some other amino acids potentiated these effects. However, others (0721, 7787) found that such effects were complicated and not readily predictable.

Pagliara and Goodman (5586) studied plasma glutamate levels in adults with gout and in control adults, and suggested that glutamate might be a substrate for over-elaboration of purines.

In 1972 Olney (5482) reviewed the difficulty of interpretation posed by normal high brain concentrations of metabolic glutamate versus the apparent high toxicity of relatively small intakes of glutamate. He saw this in terms of intracellular versus extracellular glutamate, and suggested that this might be an aspect of the blood brain barrier. He suggested that children diagnosed as minimally brain-damaged might be a minority who were susceptible to MSG, and cited the history of hexachlorophene toxicity as a possible parallel.

Glutamate and the Blood Brain Barrier

In 1950 Schwerin et al. (6568) suggested that brain glutamate levels were stabilized by the rate of its metabolism. In 1959 Lajtha et al. (4246) demonstrated continual glutamate interchange between brain and blood without increased net brain uptake when blood levels were raised.

Recently van Harreveld and coworkers (4042, 2115, 7484, 7652) applied MSG to rat cerebral cortex by electrophoresis and produced spot lesions. They predicted the topical extent of tissue changes by calculating the theoretical spread of ions by electrical forces assuming continual slow uptake by cells of vessels and tissues. They then found that a retina charged with labeled glutamate exchanged its label when stimulated with unlabeled glutamate. They also found that glutamate depolarised neurons when applied electrophoretically. They interpreted all of these findings in terms of permeability of the blood brain barrier to glutamate.

In 1972 Perez and Olney (5719) suggested that an excessive uptake of glutamate by brain might be masked by exchange or rapid metabolism.

However, a different approach by Oldendorf demonstrated that there was a clear separation of uptakes by the brain between essential and nonessential amino acids; uptake of glutamate was relatively small, and much of the brain glutamate was derived rapidly from blood glucose (8363) Then Dhopeshwarkar et al. (8359) demonstrated that

barrier differences between adult and juvenile brains vanished when a test substance was injected into the carotid artery using Oldendorf's technique; uptake, distribution, and metabolism of acetate-1-C^{14} occurred within 15 seconds. However, no reports were found of this technique being used with labeled glutamate as the test substance.

Special Studies

No published reports of mutagenic studies were found. In one unpublished report (8340) no dominant lethal mutations were found in a series of mice treated with MSG. In another (8339), host-mediated assays on intestinal bacteria of mice disclosed no mutagenicity.

Teratogenicity has been reported as slight but insignificant in chicks (4255, 4254), but significant in rabbits (7542). The rabbits were fed 25 g/kg daily for 40 days, and skeletal, gonadal, endocrine, and adipocytic deformities were reported in the litters that survived (7542). However, an unpublished report (Ajinomoto Co.) recorded no MSG teratogenicity when pregnant rabbits were given glutamic acid hydrochloride or MSG (not stated whether D- or L- form) at 25 g/kg for 15 days.

In a brief report with no details (2654), lymphocyte changes were observed after long continued high dosage of rats with MSG, but the author rejected a carcinogenic (leukemogenic) interpretation of his findings. No other studies on carcinogenicity were found.

Therapeutic Uses of Glutamate

Glutamate has been used by a number of authors to counteract or prevent ammonia convulsions with varying results (6399, 5685, 3185). It has been used to modify the circulation of rabbits with untoward results (4167). A preliminary report (5920) suggested that DL-glutamic acid HCl might be useful in epilepsy, and the possibility that glutamate might be useful in mental deficiency has been debated (0366, 7738, 8038, 7915).

Biochemical Aspects

Breakdown

No specific studies were found of the breakdown of salts of glutamic acid in the gut.

Absorption - Distribution

Blood glutamate concentrations in the rabbit were not found to be useful quantitative measures of absorption (5238). Studies in cats indicated that 2% L-glutamic acid was mostly transaminated during absorption (5238). However, stronger solutions in dogs were largely absorbed as such (5238).

A more detailed study was reported in 3-day-old piglets (8364), from which the authors concluded that plasma glutamate levels rose only when the immediate capacity of the liver to remove it was exceeded. They found plasma responses to a dose of MSG 0.1 g/kg similar to responses cited from reports on lactating women.

In humans two groups of workers concluded that the absorption of glutamic acid given separately from food

depended on other amino acids present in the food (0721, 7787). A wide variety of glutamate tolerance curves has been reported by Himwich (2996) in terms of blood levels, and he cautioned that the free acid and its salts might be absorbed at different rates. A variety of individual responses has also been reported for absorption of MSG by lactating women (8354).

Metabolism and Excretion

Rodents. Label studies have shown in mice and rats that most glutamate is converted rapidly after distribution to the organs, and that its conversions are compartmentalized (4246, 0682) and vary according to the organ and the conditions of the experiment (8033, 5932, 8349). There was a tendency under some conditions to suppress the mobilization of liver glycogen (4211), and to depress serum lipid levels (0580). Krebs cycle intermediates were influenced (3829), and there was conversion to other amino acids, especially alanine (3831). Injection of L-glutamate i.v. raised glucose levels in rabbits (2411). Brain glutamate was oxidized (7895), but the author concluded that this was metabolic glutamate owing to the blood brain barrier.

Ruminants. L-glutamate was mostly oxidized in one study (1961); in another, more was converted to carbohydrate than to protein (1960).

Pigs. Some recent comprehensive studies of glutamate metabolism have been reported (8365). Neither glutamate nor aspartate entered the CSF as such, although label was

found in glutamine, glucose, lactate, and urea in CSF. In the liver glutamate entered the mitochondria where it was metabolized by way of the Krebs cycle, and aspartate was returned to the blood. Urea was formed via α-ketoglutarate, CO_2, and carbamyl-P. Alanine was probably (8365) formed from glutamate in the blood, but the authors concluded that these conversions were greatly influenced by the form in which the glutamate had been ingested. They commented that studies with labeled carbon could be misleading, except as to the fate of the particular carbon atoms, unless methods were selected with care.

Monkeys. During pregnancy the placenta was found to maintain a higher blood level of amino acids in the fetus than in the mother (8343); the authors concluded that this relationship was suited to periods of maternal deprivation but also operated during periods of excess. Preliminary tests had revealed behavior defects in the infants, but not, as yet, neurological abnormalities (8343).

Humans. Several studies have demonstrated a serum hypolipidemic effect of glutamate (2395, 5499, 5500, 0581).

Effects on Enzymes and Other Biochemical Parameters

These have been discussed throughout preceding sections.

Drug Interactions

The additive effects of glutamate and other amino acids such as aspartate have already been noted (5492, 5482, 5486). MSG fed as 10% of the diet diminished the responses of rats to amphetamine (7915). MSG also altered the responses of rat adipose tissue cells to insulin and to norepinephrine (3859). Pyridoxine was found to enhance the action of glutamic acid or MSG in preventing cramps induced by isonicotinic acid hydrazide (3835).

In humans the emetic property of glutamate was found to be enhanced by other amino acids (4364). Some effects of glutamic acid on the circulatory system of rabbits led an author to caution against its inclusion in i.v. injection formulae for use in human patients (4761). CRS symptoms in an epileptic were thought to have been potentiated by her diphenylhydantoin therapy (7596).

Consumer Exposure

An NAS survey in 1971 (2184) reported that 75,000 tons of MSG (representing 60-70% of the poundage actually added to the nation's food supply) were produced by the firms it surveyed. Besides Oriental foods, MSG was added to frozen and canned vegetables, fresh cuts of beef, pork and veal, ground hamburger and pork, frozen or canned pork and chick products, fresh and prepared sea-food, canned meat products (such as gravies and stews), dehydrated and canned soups, salad dressings, roasted nuts, canned or frozen foods containing cheese, popcorn, and potato chips. The average usage levels ranged from 0.15% to 0.24%.

Monoammonium glutamate is used primarily in soups and with monopotassium glutamate in salt substitutes. Glutamic acid is added to baked goods, frozen dairy products, meat products, relishes, soft candies, puddings, beverages, and seasonings. The FAO/WHO Expert Committee on Food Additives met in 1971 to discuss additives in baby foods. They recommended that foods intended for infants under 12 weeks should contain no additives at all (3440). They also pointed out that even though protective detoxification mechanisms might be adequately developed after 12 weeks, young children consumed up to three times more calories per kilogram of body weight than adults. The FAO/WHO committee noted that infants and young children were often fed MSG-containing foods intended for adults, and that consequences might not appear until later in the child's development (3440).

The potential average daily intakes estimated by the NAS GRAS survey (2184) were, for infants: 0-5 months, 33.2 mg monoammonium glutamate (MAG) and 25.7 mg monosodium glutamate (MSG); 6-11 months, 3871 mg monopotassium glutamate, 2290.8 mg MAG; and 12-23 months, 5782.7 mg MAG, 714.1 mg MSG and 10.5 mg glutamic acid. It has been stated that for valid calculations of margins of safety, the natural glutamic acid content of the foods to which the various glutamate salts were added should also be taken into account (5491).

For adults, the possible average daily intake of glutamates estimated from the NAS/NRC GRAS Survey (2184) were: MSG, 1160 mg; MAG, 5267.5 mg, and glutamic acid (added) 27.5 mg. Kenney and Tidball (3676) estimated that 1 g MSG per average serving was found in many foods while as much as 40 g of glutamic acid could be ingested daily in dietary protein. Many recommendations were found that exposure to MSG should be limited until specific evidence of safety was available (3436,5485,1091,4761).

PROTEIN HYDROLYSATES

Description and Specifications

Hydrolyzed protein is a blend of naturally occurring amino acids and may exist as a liquid, paste, or powder. It occurs in a range of colors and its composition varies according to the protein source and/or processing. The powder form tends to fuse at temperatures above 95°F and is hygroscopic, requiring moisture-free handling conditions.

Acute Toxicity

The LD_{50} for administration of enzymatic casein hydrolysate by intragastric cannula to the rat has been found to be 2600 $\pm$ 1600 mg/kg BW (Boyd *et al.*, 0917) and the LD_{100} similarly determined was 2860 mg/kg BW.

Olney *et al.* (8347) have reported the results of testing various protein hydrolysate preparations by injecting mice s.c. with single doses. Two enzymatic casein hydrolysates, Cutter C.P.H. solution and Travamin (formerly called Amigen), and an acidic fibrin hydrolysate, Aminosol, were tested. The two casein hydrolysates, which were higher in glutamate content than the fibrin hydrolysate, were more potent in producing brain damage in the hypothalamus of 10-day-old infant mice, and the authors (8347) commented that margins of safety for humans were not wide.

Short-Term Studies

Olney and Ho (5492) found that three of the amino acids present in protein hydrolysates when given orally to mice

produced retinal and hypothalamic lesions.

Boyd et al. (0917) produced several toxic reactions in rats fed casein hydrolysates. The authors commented that feeding infants casein hydrolysates in amounts greater than those recommended (5 g/kg/day) might produce toxic signs.

In another study (0249) hydrolyzed plant protein fed to rats produced toxic effects when fed at 50% or 60% in the diet, but not when fed at 5% or below.

The synergistic toxicities of certain amino acids in humans were noted by Levey et al. (4364); and Smyth et al. (6878) concluded that the decreased tolerance to casein hydrolysates was probably due to the glutamic and aspartic acids which constituted one-third of the amino acid content. Smyth and co-workers (6879) also found that orally administered acid-hydrolyzed casein had an appetite depressing effect on normal men, while enzymatically hydrolyzed casein had little effect.

Special Studies

In an acute experiment in rats, increases of RNA and mitotic frequency were noted in liver cells (0186). Effects on enzymes metabolizing tyrosine and tryptophan were reported by Rosen and Milholland (6234).

Absorption

Peraino and Harper (5706) found in rats that the amino acids of hydrolysates (of zein and casein) were more rapidly absorbed into the portal blood than the amino acids of the

unhydrolyzed proteins, and that there were interactions between individual amino acids.

Steginк and Schmitt (7014) concluded after comparing blood serum amino acid levels in human infants fed two types of formula (a casein hydrolysate and a conventional cow's milk protein-based formula) that the plasma amino acid levels after feeding were largely influenced by the nature of the ingested protein source.

Metabolism

When Mital and Mathur (4989) compared feeding rats with two enzymatic protein hydrolysates (soybean and peanut) and a nutritive protein diet, they found that the soybean hydrolysate was the most rapidly absorbed through the gastrointestinal tract and was superior in promoting growth.

Ahrens and Wilson found in rats that casein was not metabolically equivalent to a mixture of its constituent amino acids.

Consumer Exposure

The protein hydrolysates used for food are largely acid- and enzyme-hydrolyzed proteins from wheat gluten, corn gluten, extracted soya flour, casein, peanut flour, yeast, dried distiller's solubles, extra cottonseed meal, fish waste, and sometimes egg albumin (2752). They are used both for flavor enhancement and for fortifying the nutritional content of a wide variety of food products including canned, frozen, or dehydrated soups, meats, and cheeses.

The degree of flavor enhancement of a particular hydrolysate

is related to its glutamate content (6063). Hydrolyzed plant proteins rich in glutamate are therefore also used to replace MSG (Table 79). For example, they are now being put in some baby and infant foods as replacements for MSG (5485). At present, no limitations have been set by the FDA on the level of their use (5689). A need for specific standards, and for the measurement of potential hazards, has been emphasized by several authors (2752, 5491,3436).

CHEMICAL INFORMATION

L-GLUTAMIC ACID

I. Nomenclature

A. Common names: L-Glutamic acid, glutaminic acid, 2-aminoglutaric acid, α-aminoglutaric acid

B. Chemical names:

2-Aminopentanedioic acid

1-Aminopropane-1,3-dicarboxylic acid

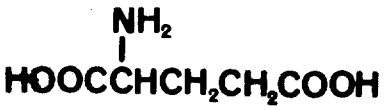
C. Trade names: Aciglut, Glutacid, Glutaminol, Glutaton

D. Chemical Abstracts Services Unique Registry Number: 6899054

II. Empirical Formula

$C_5H_9NO_4$

III. Structural Formula



IV. Molecular Weight: 147.13

V. Specifications

A. The Food Chemicals Codex Second Edition (1486) presents the following food grade specifications for glutamic acid:

1. Description

A white, practically odorless, free-flowing crystalline powder. It is slightly soluble in water, forming acid solutions. The pH of a saturated solution is about 3.2.

2. Identification

a. Dissolve about 150 mg in a mixture of 4 ml of water and 1 ml of sodium hydroxide T.S., add 1 ml of ninhydrin test solution and 100 mg of sodium acetate, and heat in a boiling water bath for 10 minutes. An intense violet-blue color is formed.

b. The glutamic acid dissolves completely on stirring when either 5.6 ml of 1 N hydrochloric acid or 6.8 ml of 1 N sodium hydroxide is added to a suspension of 1 g of the sample in 9 ml of water.

3. Specifications

Assay. Not less than 99.0 weight percent of $C_5H_9NO_4$

Specific rotation. $[\alpha]^{25°}_{546.1\ m\mu}$: Between +37.7° and +38.5°, $[\alpha]^{20°}_{D}$: Between +31.5° and +32.3°

Limits of impurities.

Arsenic (as As) - Not more than 3 ppm (0.0003%)

Chloride - Not more than 0.2%

Heavy metals (as Pb) - Not more than 20 ppm (0.002%)

Lead - Not more than 10 ppm (0.001%)

Loss on drying - Not more than 0.1%

4. Tests

Assay. Dissolve about 550 mg, accurately weighed, in 250 ml of water, add bromthymol blue and titrate with 0.1 N sodium hydroxide to a blue end point. Each milliliter of 0.1 N sodium hydroxide is equivalent to 14.71 mg of $C_5H_9NO_4$

Specific rotation. $[\alpha]_{546.1\ m\mu}^{25^\circ}$: Determine in a solution containing 11.8 g in sufficient 1.5 N hydrochloric acid to make 100 ml.

$[\alpha]_{D}^{20^\circ}$: Determine in a solution containing 10 g in sufficient 2 N hydrochloric acid to make 100 ml.

Arsenic. A sample solution prepared as directed for organic compounds meets the requirements for the arsenic test.

Chloride. Any turbidity produced by a 10-mg sample does not exceed that shown in a control containing 20 µg of chloride ion (Cl).

Heavy metals. Prepare and test a 1-g sample as directed in Method II under Heavy Metals Test, using 20 µg of lead ion (Pb) in the control.

Lead. A sample solution prepared as directed for organic compounds meets the requirements of the Lead Limit Test, using 10 µg of lead ion (Pb) in the control.

Loss on drying. Dry at 85°C for 3 hours.

Packaging and storage. Store in well-closed containers.

Functional use in food. Salt substitute; nutrient; dietary supplement.

VI. Description

A. Glutamic acid is a white, practically odorless, free-flowing crystalline powder. Orthorhombic, bispenoidal crystals from aqueous alcohol.

B. Glutamic acid has a density, d 4 (vac) = 1.538. It decomposes at 247-249°C and sublimes at 200°C (175°C at 10 Torr).

$pK_1 = 2.19$; $pK_2 = 4.25$; $pK_3 = 9.67$

Solubility in water (grams/liter)

8.64 at 25°

21.86 at 50°

55.32 at 75°

140.00 at 100°

Practically insoluble in ether, acetone, cold glacial acetic acid.

Quite insoluble in methanol and ethanol (<0.07 g/liter at 25°).

Specific rotation $[\alpha]_D^{22.4°}$ = +31.4° (c = 1.00 in 6 N HCl).

MONOAMMONIUM L-GLUTAMATE

I. Nomenclature

A. Common names: Monoammonium L-glutamate, monoammonium glutamate, ammonium glutamate, L-glutamic acid, monoammonium salt

B. Chemical names:

2-Aminopentanedioic acid, monoammonium salt, monohydrate

C. Trade name: none

D. Chemical Abstracts Services Unique Registry Number: 17140862

II. Empirical Formula

$C_5H_{12}N_2O_4 \cdot H_2O$

III. Structural Formula

IV. Molecular Weight: 182.18

V. Specifications

A. The Food Chemicals Codex Second Edition (1486) presents the following food grade specifications for monoammonium glutamate:

1. Description

A white, practically odorless, free-flowing, crystalline powder. It is freely soluble in water but is practically insoluble in common organic solvents.

2. Identification

a. To 1 ml of a 1 in 30 solution add 1 ml of triketohydrindene hydrate and 100 mg of sodium acetate, and heat in a boiling

water bath for 10 minutes. An intense violet-blue color is formed.

b. To 10 ml of a 1 in 10 solution add 5.6 ml of 1 N hydrochloric acid. A white crystalline precipitate of glutamic acid forms on standing. When 6 ml of 1 N hydrochloric acid is added to the turbid solution, the glutamic acid dissolves on stirring.

c. A 1 in 10 solution gives positive tests for ammonium.

3. Specifications

Assay. Not less than 99.0% of $C_5H_{12}N_2O_4 \cdot H_2O$

Specific rotation. $[\alpha]_{546.1\ m\mu}^{25°}$: Between +30.1° and +31.6°.

pH of a 1 in 20 solution. Between 6 and 7.

Limits of impurities.

Arsenic (as As) - Not more than 3 ppm (0.0003%).

Heavy metals (as Pb) - Not more than 20 ppm (0.002%).

Lead - Not more than 10 ppm (0.001%).

Loss on drying - Not more than 0.5%.

Residue on ignition - Not more than 0.1%.

4. Tests

Assay. Dissolve about 250 mg, accurately weighed, in 100 ml of glacial acetic acid. A few drops of water may be added prior to the addition of the acetic acid to effect faster dissolution of the sample. Titrate with 0.1 N perchloric acid in glacial acetic acid, determining the end point potentiometrically. Each milliliter of 0.1 N perchloric acid is equivalent to 9.109 mg of $C_5H_{12}N_2O_4 \cdot H_2O$.

Specific rotation. Determine in a solution containing 14.6 g in sufficient 2.3 N hydrochloric acid to make 100 ml.

pH of a 1 in 20 solution. Determine by Potentiometric Method.

Arsenic. A sample solution prepared as directed for organic compounds meets the requirements for the Arsenic Test.

Heavy metals. Prepare and test 1-g sample as directed in Method II under Heavy Metals Test, using 20 µg of lead ion (Pb) in the control.

Lead. A sample solution prepared as directed for organic compounds meets the requirements of the Lead Limit Test, using 10 µg of lead ion (Pb) in the control.

Loss on drying. Dry at 50° for 4 hours.

Residue on ignition. Ignite 1 g as directed in the general method.

Packaging and storage. Store in tight containers.

Functional use in food. Flavor enhancer; salt substitute.

VI. Description

A. A white, practically odorless, free-flowing, crystalline powder.

B. Monoammonium glutamate is freely soluble in water, practically insoluble in common organic solvents.

MONOPOTASSIUM L-GLUTAMATE

I. Nomenclature

A. Common names: Monopotassium L-glutamate, L-glutamatic acid, monopotassium salt, monopotassium glutamate, potassium glutamate, MPG

B. Chemical names:

2-Aminopentanedioic acid, monopotassium salt, monohydrate

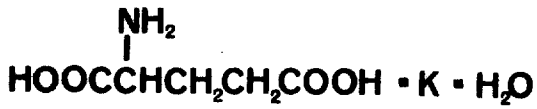
C. Trade names: none

D. Chemical Abstracts Services Unique Registry Number: 997422

II. Empirical Formula

$C_5H_8KNO_4 \cdot H_2O$

III. Structural Formula



IV. Molecular Weight: 203.24

V. Specifications

A. The Food Chemicals Codex Second Edition (1486) presents the following food grade specifications for monopotassium glutamate:

1. Description

A white, practically odorless, free-flowing, crystalline powder. It is hygroscopic, freely soluble in water, and is slightly soluble in alcohol.

2. Identification

a. To 1 ml of a 1 in 30 solution add 1 ml of triketohydrindene hydrate and 100 mg of sodium acetate, and heat in a boiling

water bath for 10 minutes. An intense, violet-blue color is formed.

b. To 10 ml of a 1 in 10 solution add 5.6 ml of 1 N hydrochloric acid. A white, crystalline precipitate of glutamic acid forms on standing. When 6 ml of 1 N hydrochloric acid is added to the turbid solution, the glutamic acid dissolves on stirring.

c. A 1 in 10 solution gives positive tests for potassium.

3. Specifications

Assay. Not less than 99.0% of $C_5H_8KNO_4 \cdot H_2O$

Specific rotation. $[\alpha]^{25°}_{546.1\ m\mu}$: Between +27.7° and +28.3°; $[\alpha]^{20°}_{D}$: Between +22.5° and +24.0°.

pH of a 1 in 50 solution. Between 6.7 and 7.3.

Limits of impurities.

Arsenic (as As) - Not more than 3 ppm (0.0003%).

Chloride - Not more than 0.1%.

Heavy metals (as Pb) - Not more than 20 ppm (0.002%).

Lead - Not more than 10 ppm (0.001%).

Loss on drying - Not more than 0.1%.

4. Tests

Assay. Dissolve about 250 mg, accurately weighed, in 100 ml of glacial acetic acid. A few drops of water may be added prior to the addition of the acetic acid to effect faster dissolution of the sample. Titrate with 0.1 N perchloric acid in glacial acetic acid, determining the end point

potentiometrically. Each milliliter of 0.1 N perchloric acid is equivalent to 10.16 mg of $C_5H_8KNO_4 \cdot H_2O$.

Specific rotation. $[\alpha]_{546.1\ m\mu}^{25°}$: Determine in a solution containing 16.3 g in sufficient 2.3 N hydrochloric acid to make 100 ml; $[\alpha]_D^{20°}$: determine in a solution containing 10 g in sufficient 2 N hydrochloric acid to make 100 ml.

pH of 1 in 50 solution. Determine by the Potentiometric Method.

Arsenic. A sample solution prepared as directed for organic compounds meets the requirements of the Arsenic Test.

Chloride. Any turbudity produced by a 20-mg sample does not exceed that shown in a control containing 20 µg of chloride ion (Cl).

Heavy metals. Prepare and test a 1-g sample as directed in Method II under Heavy Metals Test using 20 µg of lead ion (Pb) in the control.

Lead. A sample solution prepared as directed for organic compounds meets the requirements of the Lead Limit Test using 10 µg of lead ion (Pb) in the control.

Loss on drying. Dry at 60° in a vacuum for 2 hours.

Packaging and storage. Store in tight containers.

Functional use in food. Flavor enhancer; salt substitute.

VI. Description

A. The monopotassium salt of glutamic acid is a white, crystalline powder. It is free-flowing and hygroscopic.

B. Monopotassium glutamate is freely soluble in water and is slightly soluble in alcohol.

MONOSODIUM L-GLUTAMATE

I. Nomenclature

A. Common names: L-Glutamic acid, monosodium salt, sodium glutamate, monosodium L-glutamate, MSG

B. Chemical names:

2-Aminopentanedioic acid, monosodium salt, monohydrate

C. Trade names: Ajinomoto, Glutacyl, RL-50, Vetsin, Accent, Zest, Glutavene

D. Chemical Abstracts Services Unique Registry Number: 17140862

II. Empirical Formula

$C_5H_8NaNO_4 \cdot H_2O$

III. Structural Formula

IV. Molecular Weight: 187.13 (For monohydrate form shown above)

V. Specifications

A. The Food Chemicals Codex Second Edition (1486) presents the following food grade specifications for monosodium glutamate:

1. Description

White, practically odorless, free-flowing crystals or crystalline powder. It is freely soluble in water, and is sparingly soluble in alcohol. It may have either a slightly sweet or a slightly salty taste.

2. Identification

a. To 1 ml of a 1 in 30 solution add 1 ml of triketohydrindene hydrate and 100 mg of sodium acetate, and heat in a boiling

water bath for 10 minutes. An intense violet-blue color is formed.

b. To 10 ml of a 1 in 10 solution add 5.6 ml of 1 N hydrochloric acid. A white crystalline precipitate of glutamic acid forms on standing. When 6 ml of 1 N hydrochloric acid is added to the turbid solution, the glutamic acid dissolves on stirring.

c. A 1 in 10 solution gives positive tests for sodium.

3. Specifications

Assay. Not less than 99.0% of $C_5H_8NNaO_4 \cdot H_2O$.

Clarity and color of solution. Passes test.

Specific rotation. $[\alpha]^{25°}_{546.1\ m\mu}$: Between +29.7° and +30.2°; $[\alpha]^{20°}_{D}$: between +24.8° and +25.3°.

pH of a 1 in 20 solution. Between 6.7 and 7.2.

Limits of impurities.

Arsenic (as As) - Not more than 3 ppm (0.0003%).

Chloride - Not more than 0.2%.

Heavy metals (as Pb) - Not more than 20 ppm (0.002%).

Lead - Not more than 10 ppm (0.001%).

Loss on drying - Not more than 0.3%.

4. Tests

Assay. Dissolve about 250 mg, accurately weighed, in 100 ml of glacial acetic acid. A few drops of water may be added prior to the addition of the acetic acid to effect faster dissolution of the sample. Titrate with 0.1 N perchloric

acid in glacial acetic acid, determining the end point potentiometrically. Each milliliter of 0.1 N perchloric acid is equivalent to 9.356 mg of $C_5H_8NNaO_4 \cdot H_2O$.

Clarity and color of solution. A solution of 1 g of the sample in 10 ml of water is colorless and has no more turbidity than a standard mixture prepared as follows: Dilute 0.2 ml of standard chloride solution to 20 ml with water, add 1 ml of dilute nitric acid (1 in 3), 0.2 ml of a 1 in 50 solution of dextrin, and 1 ml of a 1 in 50 solution of silver nitrate, mix, and allow to stand for 15 minutes.

Specific rotation. $[\alpha]^{25°}_{546.1\ m\mu}$: Determine in a solution containing 15 g in sufficient 2.3 N hydrochloric acid to make 100 ml; $[\alpha]^{20°}_{D}$: determine in a solution containing 10 g in sufficient 2 N hydrochloric acid to make 100 ml.

pH of a 1 in 20 solution. Determine by the Potentiometric Method.

Arsenic. A sample solution prepared as directed for organic compounds meets the requirements of the Arsenic Test.

Chloride. Any turbidity produced by a 10-mg sample does not exceed that shown in a control containing 20 µg of chloride ion (Cl).

Heavy metals. Prepare and test a 1-g sample as directed in Method II under the Heavy Metals Test, using 20 µg of lead ion (Pb) in the control.

Lead. A sample solution prepared as directed for organic compounds meets the requirements of the Lead Limit Test, using 10 µg of lead ion (Pb) in the control.

Loss on drying. Dry at 60° in a vacuum for 2 hours.

Packaging and storage. Store in tight containers.

Functional use in foods. Flavor enhancer.

VI. Description

A. White or almost white, nonhygroscopic crystalline powder. The crystals are odorless to a slight peptonelike odor.

B. MSG is sparingly soluble in alcohol but is very soluble in water (42.5% by weight at 25°C; > 50% by weight at 61°C). The pH of a 5% aqueous solution is 7.0 $[\alpha]_D^{25^\circ}$: +24.2 to +25.5 (c = 8.0 in a 1.0 N HCl).

C. Stability

Does not decompose in heat processing of food. In high acid foods, MSG may be converted to glutamic acid and lose its flavor-enhancing properties (0250).

The L- form may undergo partial racemization (thus losing its flavor-enhancing properties). Racemization tends to occur under acidic and in particular, under alkaline conditions when heat promoted (0250).

Pyrrolidone formation is indicated as a problem to stability only under extremely acid conditions at high temperature (0250).

The Maillard (or browning) reaction is indicated as a problem only where the substance containing MSG is processed at high temperature in the presence of reducing sugars (0250).

Results of tests for MSG stability (0250) show that it is stable during sterilization as well as subsequent long-term storage at room temperature.

L-GLUTAMIC ACID HYDROCHLORIDE

I. Nomenclature

A. Common names: L-Glutamic acid, hydrochloride

B. Chemical names:

2-Aminopentanedioic acid, hydrochloride

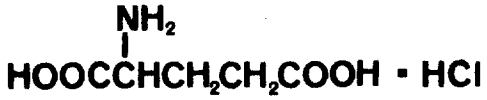
C. Trade names: Acidulin, Acidoride, Hypochylin, Antalka, Aciglumen, Pepsdol, Glutamidin, Acidogen, Gastuloric, Glutan-HCl, Glutasin, Hydrionic, Muriamic

D. Chemical Abstracts Services Unique Registry Number: 138158

II. Empirical Formula

$C_5H_9NO_4 \cdot HCl$

III. Structural Formula



IV. Molecular Weight: 183.59

V. Specifications

A. The Food Chemicals Codex Second Edition (1486) presents the following food grade specifications for glutamic acid, hydrochloride:

1. Description

A white, crystalline powder. One gram dissolves in about 3 ml of water. It is almost soluble in alcohol and ether. Its solutions are acid to litmus.

2. Identification

a. To 1 ml of a 1 in 3 solution add 1 ml of barium hydroxide solution (1 in 50), filter, and add 10 ml of alcohol. A white, crystalline precipitate of barium glutamate forms on standing.

b. To 1 ml of a 1 in 30 solution add 1 ml of ninhydrin and 100 mg of sodium acetate, and boil for 10 minutes. An intense violet-blue color is produced.

3. Specifications

Assay. Not less than 99.0% and not more than the equivalent of 101.0% of $C_5H_9NO_4 \cdot HCl$ after drying.

Specific rotation. $[\alpha]^{25°}_{546.1\ m\mu}$: Between +30.2° and +30.9°; $[\alpha]^{20°}_{D}$: between +25.2° and +25.8°.

Limits of impurities.

Arsenic (as As) - Not more than 3 ppm (0.0003%).

Heavy metals (as Pb) - Not more than 20 ppm (0.002%).

Lead - Not more than 10 ppm (0.001%).

Loss on drying - Not more than 0.5%.

Readily carbonizable substances - Passes test.

Residue on ignition - Not more than 0.25%.

4. Tests

Assay. Dissolve about 300 mg, previously dried at 80° for 4 hours and accurately weighed, in 50 ml of water, add bromthymol blue and titrate with 0.1 N sodium hydroxide. Each milliliter of 0.1 N sodium hydroxide is equivalent to 9.180 mg of $C_5H_9NO_4 \cdot HCl$.

Specific rotation. $[\alpha]^{25°}_{546.1\ m\mu}$: Determine in a solution containing 14.7 g in sufficient 0.7 N hydrochloric acid to make 100 ml; $[\alpha]^{20°}_{D}$: determine in a solution containing 10 g in sufficient 2 N hydrochloric acid to make 100 ml.

Arsenic. A sample solution prepared as directed for organic compounds meets the requirements for the Arsenic Test.

Heavy metals. Prepare and test a 1-g sample as directed in Method II under Heavy Metals Test, using 20 µg of lead ion (Pb) in the control.

Lead. A sample solution prepared as directed for organic compounds meets the requirements of the Lead Limit Test using 10 µg of lead ion (Pb) in the control.

Loss on drying. Dry at 80° for 4 hours.

Readily carbonizable substances. Dissolve 500 mg of the sample in 5 ml of sulfuric acid. The solution is colorless.

Residue on ignition. Ignite 1 g as directed in the general method.

Packaging and storage. Store in well-closed, light-resistant containers.

Functional use in food. Salt substitute; flavoring agent.

VI. Description

A. Glutamic acid, hydrochloride is a white crystalline powder, orthorhombic, bisphenoidal plates.

B. Decomposes at 214°C; nearly insoluble in alcohol and ether, 1 g dissolves in about 3 ml of water. Specific rotation $[\alpha]_D^{22°}$: +24.4° (c = 6 in water).

VII. Analytical Methods

Glutamic acid (free and as a component of hydrolyzed protein) and its derivative compounds (MSG, MKG, glutamic acid hydrochloride) are detected and estimated as glutamate following extraction and

separation from a foodstuff or tissue preparation.

A. Extraction

1. A method for extraction of free glutamic acid from fruit juices and whole fruits using 80% alcohol (where whole fruit is used, the substance is blended in a high speed blender with 80% alcohol) has been described by Fernandez-Flores et al. (2102). The amino acids extracted are isolated from the alcoholic solution by ion-exchange chromatography. The simple procedures of this method suggest its applicability to other foods, such as cereals, vegetables, or cooked foods.

2. Techniques for extracting glutamic acid (in determining MSG content) from food containing relatively large amounts of starch, sugar, spices, fats, and oils are outlined by Fernandez-Flores et al. (2101). Starch and other colloidal materials are precipitated out by using an acetone-water (1:1) extraction solvent. Removal of fats is facilitated by addition of activated carbon and subsequent filtration through a coarse-fritted glass funnel using vacuum. The amino acids are further separated using ion-exchange chromatography, where sugars are eliminated owing to their nonadsorption to the column. Salts do not interfere with the adsorption and elution of the amino acids. A small amount of dilute HCl is added in this procedure to prevent glutamate conversion to pyrrolidinone carboxylic acid. Recoveries from dried food products ranged from 93.0 to 102.0% and from liquid products from 91.6 to 105.0%.

3. Extraction of glutamic acid from nerve tissue is described by Rizzoli (6131). The tissue is homogenized in 5% trichloroacetic

acid (TCA) in the cold at a concentration of 2-3 mg tissue per 100 µl of TCA. The homogenate is then centrifuged at 23,500 g and the supernatant drawn off, capped, and kept on ice to avoid loss by evaporation or hydrolysis. Young and Lowry (8225) prepared extractions by homogenization of the tissue at 0-4°C with 10 vol. of 0.3 M $HClO_4$ and prompt neutralization with a calculated excess of 2 M $KHCO_3$. After allowing the CO_2 to come off, the supernatant fluid was removed from the $KClO_4$ precipitate.

Where enzymatic methods for analysis of glutamate are to be used, Graham and Aprison (2632) suggest that tissue extraction be done using cold 75% alcohol because both TCA and $HClO_4$ may inhibit the enzymes involved in these assays.

4. Methods for extraction of free amino acids from blood plasma are described by Stein and Moore (7019). The extracted blood is centrifuged in heparinized tubes and the plasma deproteinized by equilibrium dialysis, ultrafiltration, or precipitation with picric acid. The free amino acids in the deproteinized solution may then be separated by ion-exchange chromatography which also removes excess picric acid.

B. Separation and Isolation

1. Glutamic acid can be separated from a mixture of free amino acids containing other dicarboxylic acids by paper chromatographic methods. R_f values for glutamic acid and similar amino acids in two different solvent systems are shown in Table 1.

Bailey et al. (0446) have demonstrated a paper chromatographic method of separation for detection of MSG using n-butanol-glacial acetic acid-distilled water (12:3:5) as a solvent on Whatman 3 MM chromatographic sheets. The spots

Table 1 (0475)

R_f Values of Glutamic Acid, Aspartic Acid, and Their Amides

	Solvents[a]	
	A	B
Aspartic	0.19	0.19
Asparagine	0.40	0.27
Glutamic	0.31	0.34
Glutamine	0.57	

[a]Solvent A: Phenol salt with H_2O and crystal HCN [Consden et al., Biochem. J., 38:224(1944), quoted in 0475].
Solvent B: Isobutyric acid: H_2O (4:1) [Berry, Metabolism, 9:373 (1960), quoted in 0475].

Following separation, the amino acids can be detected by spraying with ninhydrin [Spackman et al., Anal. Chem., 30:1190 (1958), quoted in 0475].

were colored with ninhydrin and the R_f value was found to be 0.284. The method was sensitive to 1 μg of MSG and the recovery was 100% (S.D. = 4%).

2. Stein and Moore (7018) describe a method for isolation of glutamic acid from a mixture of amino acids by starch column chromatography. Greatest resolution of glutamic acid from other amino acids was obtained using a solvent mixture prepared as tert-butyl alcohol-sec-butyl alcohol-0.1 N HCl (2:1:1). Besides the increased resolving power, this solvent showed no tendency toward esterification of glutamic acid, as evidenced by a mean recovery of 98.1% of a 0.426 mg load of glutamic acid. A single chromatogram required about 2.5 mg of total amino acid mixture and collection of fractions over several 24-hour days.

3. Rosenblum and Wolfmann (6243) have separated glutamic acid and other dicarboxylic amino acids from the basic and neutral amino acids using anion-exchange chromatography. The resin employed was Rohm and Haas 100-200 mesh CG-4B (weakly basic) equilibrated to pH 4.5 with 0.2 N sodium acetate buffer. The sample (also at pH 4.5) was loaded on the column and eluted with 0.2 N sodium acetate. Recovery of glutamic acid from standard solutions was 100% ± 5% and analysis by AutoAnalyzer has shown sensitivity for 0.06-1 μg of glutamic acid.

An anion-exchange method using Amberlite IR 4 resin gave complete separation of glutamic acid from an amino acid mixture though recovery was not quantitative (about 91%) (1489).

4. Cation-exchange chromatography is used commonly in amino acid separation and employs resins such as Dowex 50W-XS (H+ form).

C. Quantitative Analytical Methods

1. Gas-liquid chromatography has been used to analyze free amino acids.

In experiments where extracts of fruits and fruit juices were analyzed, the sensitivity was less than 0.5 mg per 100 g of the original sample (2102).

2. Glutamic acid when isolated from other acids can be quantitated using the Sorensen formol titration method. The sample, neutralized to pH 7, is mixed with 37% formaldehyde and neutralized to pH 7 with 0.1 N NaOH. The mixture is titrated to pH 8.9 with 0.1 N NaOH using a pH meter. A blank consisting of formaldehyde and 1 N HCl (neutralized to pH 7) is also titrated to pH 8.9. The calculation is:

% glutamic acid in sample = (ml NaOH used in titration - blank)

X N X 0.147 X 100/wt of original sample

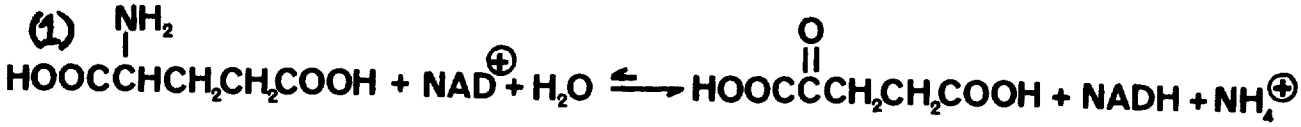
(% MSG = % glutamic acid X 1.15).

3. Among the most widely known methods for the qualitative detection of α-amino compounds is the ninhydrin reaction. Under rigorously adhered-to procedures, the method may also be used for quantitative work (5059). The colored product formed by the reaction of ninhydrin with the α-NH_2 groups is used as the basis for this spectrophotometric determination of amino acids. the use of hydrindantin or stannous chloride to eliminate interfering oxidative side reactions has rendered the assay reproducible. Using reaction conditions of pH 5 and 100°C, the accuracy is 2% for samples of individual amino acids containing 2.5 μg of α-NH_2 nitrogen. The absorption maximum for the blue-colored product is 570 mμ (5059).

4. The isotope dilution method, theoretically applicable to all amino acids, is described by Barker et al. (0518) for the quantitation of glutamate. Assays have been shown to give values near 99.7% of the theoretical, but the equipment is seldom available.

In the above chromatographic isolation techniques, there is no separation of D- and L- forms of glutamic acid (0475,7018), nor do the formol titration, ninhydrin, and GLC methods of quantitation make this separation. Further, the formol titration and ninhydrin methods require that the glutamic acid sample be free of any other amino acids to give accurate results. The following enzymatic and microbiological methods of quantitation offer specificity for the L-isomer of glutamic acid as well as some circumvention of the absolute isolation requirement of glutamic acid from other amino acids.

5. A commonly used enzymatic method for glutamate determination is that employing glutamate dehydrogenase to convert the glutamate to α-keto-glutaric acid and in the process reduce NAD+ to NADH as:



(1) $HOOCCH(NH_2)CH_2CH_2COOH + NAD^{\oplus} + H_2O \rightleftharpoons HOOCC(=O)CH_2CH_2COOH + NADH + NH_4^{\oplus}$

Reaction (1) has an equilibrium far to the left; therefore steps must be taken to render the reaction usable for assays.

a. Sowerby and Ottaway (6940) have attempted to drive the reaction by transferring the hydrogen of NADH to either 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) or 3-(4,5-dimethylthiazol-1-yl)-2,5-phenyl tetrazolium bromide (MTT). The transfer is catalyzed by N-methyl-phenazine methosulfate (PMS). The glutamate is estimated by reading optical density at 460 mμ (INT, Beer's law obeyed up to about 0.2 μmoles of glutamate or at 560 mμ (MTT, Beer's law obeyed up to about 1 μmole of glutamate). Although this method could estimate amounts of 0.3 μmole of glutamate or less, it was not stoichiometric; nevertheless, glutamine could also be estimated with the use of part-purified glutaminase (6940).

b. Another method of driving the reaction is to remove the α-ketoglutarate by reaction with hydrazine and subsequent determination of glutamate by assaying NADH produced through fluorometric or optical density (OD_{340}) methods. Graham and Aprison (2632) have used this method to detect 1 x 10^{-10} moles of glutamate with a coefficient of variation less than 2%.

Optimum conditions for the glutamate dehydrogenase method have been reported (6940) as pH 7.6 (at pH 9.6 acids other than glutamate are oxidized), and temperatures between 30 and 37°C to trap α-ketoglutarate.

The use of hydroxylamine and second reaction sequence methods for reoxidation of NADH to drive the glutamic dehydrogenase reaction have also been reported (0475).

6. Two other enzymatic methods, the glutamate decarboxylase and the coupled transaminase reactions, are discussed by Balis (0475) as a means of glutamate quantitation.

7. Snell (6881) has described methods of assay for glutamate using lactic acid bacteria and measurement of culture growth as a means of quantitating glutamate concentrations. Measurement is made directly as increase in optical density of the culture medium or by assay of acid produced. General precautions are advised: the assay medium is prepared so that addition of the test sample does not alter the medium quantitatively or qualitatively except with respect to the substance being assayed. Suggested media for lactic acid bacteria determination of amino acids are shown in Table 2.

Specific problems of glutamic acid assay involve nonquantitative growth when low concentrations of glutamic acid are being measured. Investigation has suggested that this is due to needed conversion of at least some of the glutamic acid to glutamine before it can be utilized. Lyman et al. (Abstr.

Table 2. Suggested Media for Use in Determining Amino Acids with Lactic Acid Bacteria (6881)

Substance	Concentration per 10 ml of Final Medium[a]								
	b	c	d	e	f	g	h	i	j
Glucose, mg	100	100	200	100	175	100	100	100	200
Sodium acetate, mg	60	60	72.5	60	87.5	60	60	60	200
KH_3PO_4, mg	5	5	5	10	5	5	5	5	5
K_2HPO_4, mg	5	5	5	10	5	5	5	5	5
$MgSO_47H_2O$, mg	2	2	2	2	2	2	2	2	2
$FeSO_47H_2O$, mg	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
$MnSO_44H_2O$, mg	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
NaCl, mg[k]	0.1	0.1	0.1	0.1	0.1	0.1		100	0.1
$(NH_4)_2SO_4$, mg				30	30				
Thiamine hydrochloride, mg%	1.0	1.0	1.0	1.0	4.0	10	10	5.0	2
Pyridoxin hydrochloride, mg%	1.0	1.0	1.0	3.0	4.0	2.0	16	5.0	2
Calcium pantothebate, mg%	1.0	1.0	1.0	1.0	4.0	2.0	10	1.0	2
Riboflavin, mg%	2.0	2.0	2.0	1.0	4.0	2.0	20	2.0	4
Nicotinic acid, mg%	2.0	2.0	4.0	1.0	4.0	4.0	20	5.0	2
Biotin, mg%	0.004	0.004	0.004	0.002	0.004	0.05	0.05	0.1	1
p-Aminobenzoic acid, mg%	1.0	0.1	0.005	3.0	4.0	2.0	1.0	0.1	0.002
Folic acid[l]				0.1		0.11	0.04		
Choline chloride, mg%				25					
Inositol, mg%				25					
Adenine, mg	0.1	0.1	0.05	0.1	0.04	0.5	1.0	0.1	0.1
Guanine, mg	0.1	0.1	0.05	0.1	0.04	0.5	1.0	0.1	0.1
Uracil, mg	0.1	0.1	0.05	0.1	0.04	0.5	1.0	0.1	0.1
Xanthine, mg				0.1	0.04				
Aspartic acid, mg		1.6	4.0	4.0	0.83	1.0	g		4.0[d]
Glutamic acid, mg		2.0	4.0	10.0	2.5	2.0	g		4.0
Arginine monohydrochloride, mg		0.1	2.0	1.0	0.83	2.0	0.50		0.5
Histidine monohydrochloride, mg			2.0	1.0	0.83	2.0	0.50		0.5

Table 2 (continued).

Substance	Concentration per 10 ml of Final Medium[a]								
	b	c	d	e	f	g	h	i	j
Lysine monohydrochloride, mg		0.4	2.0	1.0	0.42	1.0	1.0		2.0
Alanine, mg		1.0	1.0	1.0	0.42	1.0	1.0		1.0
Cystine, mg	1.0	0.10	2.0	1.0	0.83	2.0	1.0	1.0	1.0
Glycine, mg				1.0		2.0	1.0		
Hydroxyproline, mg				1.0	0.83	2.0	1.0		
Isoleucine, mg		0.50	1.0	1.0	0.42	1.0	1.0		1.0
Leucine, mg		0.50	1.0	1.0	0.83	2.0	2.0		1.0
Methionine, mg		0.10	1.0	1.0	0.42	1.0	0.50		0.5
Norleucine, mg				1.0	0.42	1.0	0.5		
Norvaline, mg				1.0					
Phenylalanine, mg		0.10	2.0	1.0	0.42	1.0	0.50		0.5
Proline, mg			2.0	1.0	0.83	2.0	1.0		
Serine, mg			1.0	1.0	0.42	1.0	0.25		
Threonine, mg		0.50	1.0	1.0	0.42	1.0	1.0		1.0
Tryptophan, mg	b	0.10	2.0	1.0	0.83	2.0	0.50	1.0	0.4
Tyrosine, mg		0.10	1.0	1.0	0.83	2.0	0.20		0.4
Valine, mg		0.50	1.0	1.0	0.42	1.0	1.0		1.0
Hydrolyzed casein, mg	50							50[i]	

[a]No convention has been followed by different investigators concerning use of the l- or dl- forms of the amino acids. All comparisons in this table are on the basis of the weight of the naturally occurring l-isomer added. If dl-amino acids were used, the amount added was twice that indicated. This procedure seems justified, since in most cases so far investigated, the unnatural isomers of the amino acids have not been available for growth to any marked extent.

[b]Recommended for assay of tryptophan with L. arabinosus. Tryptophan is omitted when medium is used for this purpose. The medium is the same as that recommended by Snell and Wright (1941) [see original paper for references] for nicotinic acid assay, except for addition of p-aminobenzoic acid and nicotinic acid, and omission of tryptophan.

Table 2 (continued).

[c]Media similar to this have been used with L. arabinosus for assay of some amino acids, e.g., valine and leucine, by omitting the proper one from the medium (cf. Schweigert et al., 1944).

[d]Recommended for assay of leucine, isoleucine, or valine with L. arabinosus by omitting the proper amino acid. The authors also add a tomato juice eluate factor at a level of 1 mg per 10 ml of medium. Some evidence indicates this may be replaced by a folic acid concentrate (cf. Baumgarten et al.), although the active principle does not appear to be folic acid.

[e]Recommended for assay of arginine and valine with either L. casei or L. arabinosus, but devised to support growth of all lactic acid bacteria whose nutritional requirements are known, and thus to be generally applicable as a base medium for assay of other amino acids by omitting proper amino acid from the medium.

[f]Used as a base for determining amino acid requirements of L. arabinosus, and used for tentative determination of leucine, valine, and phenylalanine.

[g]Devised as a complete medium allowing maximum growth of L. arabinosus and L. casei, for later use in determining amino acids.

[h]Devised for determination of glutamic acid with L. arabinosus. 4.0 mg asparagine is added per 10 ml in place of aspartic acid.

[i]Devised for determination of glutamic acid with L. arabinosus. The casein hydrolyzate used has been freed from glutamic acid by converting the latter to pyrrolidone carboxylic acid and extracting with ethyl acetate.

[j]Recommended for determination of valine and leucine in both protein hydrolyzates and fresh foodstuffs. When thus used, the appropriate amino acid is omitted from the medium. Test organism: L. arabinosus. Asparagine added in place of aspartic acid.

[k]Most media contain more NaCl than is indicated by these additions due to use of HCl to dissolve or preserve certain constituents of the medium, and subsequent neutralization.

[l]Expressed as micrograms of material 40,000 times as active by weight as an arbitrary standard, liver fraction B. 0.1 mg% of material of this potency is about 20 times the amount required to produce maximal growth of L. casei on a folic acid-free medium. Very crude concentrates of this material are suitable for use.

108th mtg. American Chemical Society, 1944, quoted in 6881) added glutamine to their medium (see Table 2, glutamic acid omitted) in an amount sufficient to initiate growth but insufficient to affect more than a fraction of total growth requirement, and obtained results which gave a growth curve proportional to glutamic acid even when low concentrations were used. The results obtained with rigorously controlled conditions are shown and compared to other analytical methods in Table 3 (see original paper for assay references).

However, Kiuken et al. [Science 98: 273 (1943), quoted in 6881] reported that DL-glutamic acid was only 50% as active as the L-(+)-form.

VIII. Occurrence

Plants and Animals

Glutamic acid is stated to comprise about 20% of total amino acids found in natural protein sources. Free glutamic acid occurs naturally in a wide variety of vegetables, and in fish and meat products in the range of 0.005-0.23% (4862). Wheat protein is 29% glutamic acid and corn protein 25% (5116). Glutamic acid is lost during storage or upon refrigeration; thus young, freshly harvested vegetables have a higher glutamic acid content and fresher flavor than mature vegetables. Raw vegetables can lose 25-35% of their glutamic acid during the first 24 hours of refrigerated storage (2727).

Several workers have attempted determinations of the amounts of glutamic acid occurring naturally, but these are not easily made. Muller (5116) used paper electrophoresis to separate free glutamic acid out of foods. Although this method did not distinguish between the D and L forms of the acid, the author felt that since only the L form was contained in the systems studied, the discrimination was not necessary. Unbound glutamic acid was found as a salt. Muller's findings are reproduced in Tables 4-10. He noted that the estimations

Table 3. Glutamic Acid Content of Various Substances: Comparative Values (6881)

Preparation	Microbiological Assay[a] Dunn et al. (1944) %	Lewis, Olcott (1945) %	Lyman et al. (1945) %	Hac et al. (1945) %	Chemical Procedures
Casein	21.2	19.7	21.5	21.1	--
Casein, corrected for ash	22.5	--	22.4	--	22.0 (Bailey, Chibnall et al., 1943) 22.0 (Olcott, 1944)
Silk fibroin	2.03±0.05	--	--	--	--
Egg Albumin	--	11.6-13.7[b]	14.3	14.3-15.1[b]	16.0 (Chibnall et al., 1943)
Gliadin	--	44.2	--	44.9	45.7 (Olcott, 1944) 46.9 (Bailey, Chibnall et al., 1943)
Lactoglobulin	--	18.7	--	19.0[c]	21.5 (Chibnall et al., 1943) 21.5 (Olcott, 1944)
Gelatin	--	10.2, 10.8	--	10.2	11.7 (Olcott, 1944)
Brewer's yeast	--	--	5.56	--	--
Torula yeast	8.0	--	--	--	--

Table 3. Glutamic Acid Content of Various Substances: Comparative Values (Cont'd)

[a] All investigators have used L. arabinosus as the test organism; media are formulated in Table 2. Assay range: In media unsupplemented with glutamine, range varies with size of inoculum and other factors, but is usually between 0 and 250 mg% L(+) glutamic acid per 10 ml medium. With glutamine added to initiate growth the range is 0-150 mg%.

[b] Interfering substances were noted in egg albumin, not present in most other samples, which contributed to variability in these assays.

[c] Values of 19.0 and 19.1% were recently reported for the glutamic acid content of β-lactoglobulin by the isotope dilution procedure (Foster, J. Biol. Chem., 159: 431, 1945).

Table 4. Fresh Vegetables (5116)

No.	Designation	% Glutamate of the CB[a]	% DS[a]	% Glutamate of the DS[a]
1	Beans, fresh	0.002	8.8	0.02
2	Beans, frozen	0.008	8.6	0.09
3	Peas, frozen	0.076	20.6	0.37
4	Carrots, frozen	0.013	9.6	0.14
5	Leeks, frozen	0.016	8.8	0.18
6	Potatoes	0.016	25.3	0.24

[a] CB, commercial base; DS, dry substance.

Table 5. Dried Vegetables (5116)

No.	Designation	% Glutamate by CB
1	Mushroom powder	0.18
2	Lump mushrooms	0.20
3	Mushroom	0.22
4	Mushroom	1.7
5	Paprika, red	0.06
6	Paprika, green	0.05
7	Carrots	0.07
8	Carrot juice	0.12
9	Peas	0.33
10	Beans	0.11
11	Leeks, green	0.08
12	Leeks, powdered	0.11
13	Leeks, white	0.12
14	Celery (ground bulbs)	0.29
15	Celery leaves	0.05
16	Diced onions	0.10
17	Tomato flakes	1.50
18	Dried potatoes	0.13

Table 6. Dried Herbs (5116)

No.	Designation	% Glutamate by CB
1	Rose paprika	0.15
2	Paprika, precious sweet (edelsuss)	0.10
3	Cayenne pepper	0.12
4	White pepper	0.01
5	Black pepper	0.04
6	Marjoram	0.03
7	Garlic, dried	0.13
8	Onion juice	0.03
9	Curry	0.03
10	Parsley	0.06
11	Thyme	0.04
12	Rosemary	<0.01
13	Pimento	<0.01
14	Bay leaves	<0.01
15	Lovestock (liebstockel)	0.07
16	Caraway	0.11
17	Coriander	0.07
18	Nutmeg	<0.01
19	Clove	<0.01
20	Celery seed	0.04

Table 7. Fruits and Juices (5116)

No.	Designation	% Glutamate of the CB	% DS	% Glutamate of the DS
1	Orange	0.023	6.5	0.355
2	Grapefruit	0.036	11.0	0.327
3	Apple, "Cox-Orange"	0.004	14.7	0.027
4	Apple, "Boskop"	0.003	16.8	0.015
5	Currant, black	0.003	13.7	0.022
6	Tomato juice	0.27	5.3	5.1
7	Tomato juice, concentrated	0.37	6.3	5.9

Table 8. Meat and Fish (5116)

No.	Designation	% Glutamate of the CB
1	Calf, raw	<0.01
2	Calf, cooked	<0.01
3	Beef, raw	<0.01
4	Beef, cooked	<0.01
5	Pork, raw	<0.01
6	Pork, cooked	<0.01
7	Meat extract	0.25
8	Fresh herring	<0.01
9	Cod fish filet, frozen	<0.01

Table 9. Various Staples of Life (5116)

No.	Designation	% Glutamate of the CB	% DS	% Glutamate of the DS
1	White bread	0.002	65.2	0.003
2	Whole corn bread	0.009	58.5	0.016
3	Rye bread	0.025	61.7	0.041
4	Whole corn-oat flakes	0.014	90.5	0.016
5	Poultry egg	0.023	25.1	0.092
6	Fresh milk	0.004	13.9	0.027
7	Condensed milk	0.014	33.4	0.042

Table 10. Cheese (5116)

No.	Designation	No. of Samples	% Fat	% Glutamate of the CB	% DS	% Glutamate of the DS
1	Cottage (skim curd)	1	20	0.015	18.3	0.081
2	Fresh cheese	3	70	0.004-0.006	41.4-47.1	0.008-0.014
3	Melted cheese (cheese spread)	4	60	0.046-0.193	47.6-48.5	0.089-0.405
4	Melted cheese	3	45	0.033-0.257	45.6-51.1	0.072-0.503
5	Melted cheese	2	30	0.190-0.204	35.7-36.8	0.516-0.571
6	Parmesan	3	32-35	1.803-2.17	85.7-86.8	2.14-2.43
7	Gouda	7	—	0.113-0.979	54.9-58.0	0.206-1.75
8	Emmentaler	3	—	0.792-0.908	63.3-67.8	1.18-1.39
9	Edam	1	--	0.075	53.3	0.141
10	Tilsit	1	—	0.322	61.1	0.527

were only approximately accurate because the quantity of soluble glutamic acid was influenced by brand, growth conditions, and storage time and conditions.

Fernandez-Flores et al. (2102) used gas-liquid chromatography to determine the glutamic acid content of some fruits. Amounts in milligrams per 100 g fruit were: apples 0.9, avocado 13.6, banana 36.7, blackberry 17.4, cantaloupe 54.3, watermelon 29.1, pineapple 18.4-6.9 (varies according to species), white grapes 35.0, and black grapes 43.6.

Maeda et al. (4537), using a complicated microbiological quantitative analysis, determined the glutamic acid content of a variety of sea foods, meats, and vegetables. Their findings are shown in Table 11. The free glutamic acid content of shellfish and chicken was high, while the beef analyzed had a low amount. Tomatoes, mushrooms, squash, and sweet potatoes were also high in glutamic acid.

The main sources of commercial glutamic acid or monosodium glutamate for flavoring purposes have been, in the Orient, Laminaria japonica, a seaweed; and a soy sauce produced by enzymatic digestion of soy beans [soy protein is about 20% glutamic acid (4862)]. In the United States glutamic acid was initially extracted from Steffen's waste liquor, a by-product of sugar beet extraction that contained a high percentage of glutamate compound. Wheat gluten, which contains approximately 40% glutamic acid, is the other major source of commercial glutamic acid in this country, and is probably the richest and most economical source, although corn gluten is also used (4862).

Mice

Olney (5481) estimated that the central nervous system of the mouse contained approximately 10 μmoles/g free glutamic acid, "considerably" more than any other amino acid.

Table 11. Free Glutamic Acid Content in Foods (4537)

Sample	Water content	Free glutamic acid content: Within the sample — Glutamic acid (mg%)	Within the sample — Amino-N (mg%)	Converted to a dry state — Glutamic acid (mg%)	Converted to a dry state — Amino-N (mg%)	pH	Remarks
Seafood (fish)							
Sardine	63.8	280	27	880	85	6.15	Head, tail and bones removed
Carp	75.0	12	1.1	48	4.4	6.33	Sliced raw
Prussian carp (*Cyrpinus auratus*)	76.5	13	1.2	55	5.1	6.61	(Including the tail)
Eel	62.2	10	0.95	27	2.6	6.53	Head, tail, intestines and bones removed
Mackerel	53.8	36	3.4	78	7.4	6.12	Head, tail and bones removed
Horse mackerel	74.6	19	1.8	75	7.1	6.37	Head, intestines, bones, tail and scales removed

Table 11. Free Glutamic Acid Content in Foods (Cont'd)

Sample	Water content	Free glutamic acid content: Within the sample — Glutamic acid (mg%)	Within the sample — Amino-N (mg%)	Converted to a dry state — Glutamic acid (mg%)	Converted to a dry state — Amino-N (mg%)	pH	Remarks
Tuna	77.3	8.9	0.85	39	3.7	5.95	A quantity equivalent to a whole fish taken from the anterior and middle section of the posterior third
Tuna	73.6	5.3	0.50	20	1.9	6.30	"
Sea bream	78.1	9.3	0.89	42	4.0	6.47	Sliced raw (including the skin)
Blowfish	80.3	6.8	0.65	35	3.3	5.87	Flesh
Turbot	78.1	38	3.6	174	16.4	6.45	Head, tail, bones, intestines and skin removed
Seafood (Crustaceans)							
Prawn	75.1	43	4.1	173	16.5	7.05	Vein removed
	76.1	51	4.9	210	21	7.17	
Clams	88.7	41	3.9	360	35	6.51	Shucked

Table 11. Free Glutamic Acid Content in Foods (Cont'd)

Sample	Water content	Free glutamic acid content: Within the sample — Glutamic acid (mg%)	Within the sample — Amino-N (mg%)	Converted to a dry state — Glutamic acid (mg%)	Converted to a dry state — Amino-N (mg%)	pH	Remarks
Ark-shell	85.6	151	14.4	1050	100	6.63	Shucked
Corbicula	89.3	23	2.2	214	20.4	6.83	Shucked
Short neck clam	86.1	136	12.9	980	93	6.51	Shucked
Abalone	80.3	72	6.9	370	35	6.00	Shucked
Oyster	81.0	137	13.0	720	69	6.17	Shucked
Seafood (Cephalopods)							
Sagittated calamary	77.5	146	13.9	650	62	6.03	Entrails removed
Meat							
Beef tenderloin	75.9	33	3.1	140	13.1	5.55	1 head
Beef round	75.5	11	1.0	45	4.3	5.47	

Table 11. Free Glutamic Acid Content in Foods (Cont'd)

Sample	Water content	Free glutamic acid content: Within the sample — Glutamic acid (mg%)	Within the sample — Amino-N (mg%)	Converted to a dry state — Glutamic acid (mg%)	Converted to a dry state — Amino-N (mg%)	pH	Remarks
Pork tenderloin	72.0	23	2.2	82	7.8	5.85	1 head
Chicken meat	75.6	44	4.2	180	17.1	5.97	1 fowl
Chicken bone	48.6	40	3.8	78	7.4	7.82	1 fowl
Vegetables (fruits and vegetables)							
Eggplant	93.7	16	1.5	250	24	5.60	Stem removed
Tomato (unripe)	95.0	58	5.5	1160	110	3.89	Stem removed
Tomato (ripe)	93.9	140	13.3	2280	217	4.10	Stem removed
Cucumber	95.4	23	2.2	500	48	5.85	Both ends removed
White musk-melon	96.1	16	1.5	410	39	5.78	Both ends and the seeds removed

Table 11. Free Glutamic Acid Content in Foods (Cont'd)

Sample	Water content	Free glutamic acid content: Within the sample — Glutamic acid (mg%)	Within the sample — Amino-N (mg%)	Converted to a dry state — Glutamic acid (mg%)	Converted to a dry state — Amino-N (mg%)	pH	Remarks
Squash	92.9	47	4.5	660	63	5.83	Stem and seeds removed
Green soybean	71.1	19	1.8	66	6.3	6.87	Husk and membrane removed
Leafy vegetables							
Cabbage	93.3	37	3.5	550	52	6.14	Stem removed
Onion	92.1	18	1.7	228	22	5.13	Both ends and membranes removed
Spinach	91.5	39	3.7	460	44	6.11	Root removed
Edible roots							
Bermuda onion	91.7	21	2.0	250	24	5.17	Skin and root removed
Carrots	89.1	33	3.1	300	29	5.88	
Potatoes							
Sweet potato	66.4	60	5.7	179	17.0	6.22	

Man

Human plasma contains 4.4-4.5 mg/liter of free glutamic acid and 0.9 mg/100 ml of bound glutamic acid. Human urine contains 2.1-3.9 µg/mg of free glutamic acid to creatinine and 200 µg/mg bound glutamic acid to creatinine (5741). Human spinal fluid contains 0.34-1.64 (mean 1.03 mg/liter) free glutamic acid (1789). Human milk contains 1.2% protein with 20% of the protein being bound glutamic acid, equivalent to 3 g/liter calculated as sodium glutamate. Free glutamic acid is 300 mg/liter (3441).

In 1954 Himwich and Peterson (3001) found the basal plasma glutamate level to be highly variable in and between adult humans. Typical fluctuations over the course of a year were 0.6-4.6 (average 1.9) mg/100 ml, and 1.0-4.8 (average 3.8) mg/100 ml. A test dose of 15 g MSG by mouth raised the plasma glutamate level by at most 30 mg/100 ml, after an interval that ranged 60-100 minutes, and the level returned to normal usually within 180 minutes from the dose. The amount of the increases varied tenfold among individuals.

No reports of glutamate tolerance curves in children have been found.

BIOLOGICAL DATA

I. Acute Toxicity

A number of lethal-dose (LD) studies are summarized in Table 12.

W. Pinto-Scognamiglio et al. (5804) studied the acute toxicity of MSG when administered orally to mice and rats (number, sex, strain, and age not stated). The doses used to establish the toxic values shown in Table 12 were not given.

When Klingmuller and Vogelsang (3829) administered more than 3.6 g/kg MSG intraperitoneally (i.p.) to rats weighing 100-150 g, more than 50% died with clonic seizures within 100 minutes, with an occasional death occurring after 12 hours. The same researchers (3829) found that 0.09 g/kg glutamic acid, neutralized with ammonium hydroxide, was sufficient to elicit a picture of poisoning characterized by typical Kussmaul respiration, often beginning before the seizure and followed by loss of consciousness. At a slightly higher dosage of monoammonium glutamate (1.0 g/kg) more than 50% of the animals died in seizure or in acidotic coma. Ammonium chloride produced similar results but was judged less potent. Further experiments in rabbits (3829) showed a rapid transient increase of serum glutamate and an apparent conversion of glutamate to α-ketoglutarate after injection of MSG 0.3 g/kg intravenously (i.v.) or 0.5 g/kg i.p. However, there was also a corresponding decrease in serum pyruvate, and after some in vitro experiments the authors concluded that transamination of MSG occurred in the blood as well as in the liver. They speculated that glutamine or glutamate entered the cerebrospinal fluid and, once there, might facilitate binding or transport of ammonium.

Carew and Foss (1155) determined the toxicity of single doses of an aqueous solution of MSG administered subcutaneously (s.c.) in the upper part of the

Table 12

Acute Toxicity

Substance	Animal	Sex	No.	Route	Dosage mg/kg body wt	Measure-ment	Ref.
MSG	Mouse	Not stated		Oral	1920(2284-1613)[a]	LD_{50}	Pinto-Scognamiglio, W. et al. (5804)
MSG	Rat	Not stated		Oral	1660(1890-1450)[a]	LD_{50}	Pinto-Scognamiglio, W. et al. (5804)
MSG	Rat	Not stated		i.p.	3600	LD_{50}	Klingmuller and Vogelsang (3829)
MAG	Rat	Not stated		i.p.	1000	LD_{50}	Klingmuller and Vogelsang (3829)
Enzymatic casein hydrolysate	Rat	M	20	Intragastric Cannula	2600 ± 1600	LD_{50}	Boyd et al. (0917)
Enzymatic casein hydrolysate	Rat	M	20	Intragastric Cannula	2860	LD_{100}	Boyd et al. (0917)
MSG	Chickens	Not stated		s.c.	3000-4000	LD_{50}	Carew and Foss (1155)
MSG	Chickens	Not stated		s.c.	5000	LD_{100}	Carew and Foss (1155)

[a]LD_{50} mg/kg with limit of confidence calculated up to 95%.

neck of day-old broiler chicks. At 2 mg/g BW, there was a significant reduction of body weight. Higher doses were lethal. See p. 99.

II. Toxic Effects: Retinal Lesions

Doses of MSG that have been reported to produce lesions of the inner retina are shown in Table 13. In some studies lower dosages failed to produce such lesions.

Table 13. Lesions of the Inner Retina

Route	Dose g/kg	No. of doses (once daily)	Animals	References
i.v.	0.1	50	rabbit	3862
s-c	4.8	1	mouse	4487
	2.2-4.0	1-10	mouse	1432, 5481
	2.2	15	rat	2804
i-p	2.2	10-18	mouse, rat	2235, 5878
	0.5-2.0	16	rabbit	2770
	4.0	1	chick	4025

A. Mice

1. In 1957, Lucas and Newhouse (4487) reported for the first time that a degenerative lesion was produced in neonatal mouse retina following s.c. administration of monosodium L-glutamate (MSG). This toxic effect was reported one year before MSG was made GRAS (Schaumburg and Byck, 1091).

Seventy suckling Strong A2(Glaxo) mice aged 2-16 days were injected s.c. with a 0.01-0.20 ml solution of MSG in Krebs-Ringer solution according to the dosage schedule shown in Table 14 (4487).

Forty-one female adult Strong A2(Glaxo) mice from an inbred colony, weighing 15-36 g, six of which were mothers of litters discussed in Table 15,

Table 14. Dosage of Substances Given Daily to Mouselings aged 2-16 days (4487)

Age, days and weight (g)[a]	MSG (mg/kg BW)
2 (1.80)	2.20
6 (3.28)	3.70
10 (4.36)	4.60
13 (5.43)	4.80
16 (5.50)	5.40
Total dose (mg)[b]	240
Animals	
No. used	70
Survivors for hist. study, No.	39
Age when killed, days	2-40

[a] Mean value based on 5 litters comprising 27 mouselings.

[b] Typical total dose given between 2 and 16 days.

Table 15. Dosage of MSG Administered Subcutaneously to Adult Mice (4487)

Dosage[a] (mg/g BW)	No.	Survivors examined histologically No.	Time after last dose (days)
4.0	3	3	
6.0	9	7	1 hr - 21 days
8.0	12	7	
10.0	3	--	
3.0-4.0[b]	8	8	28
1.0-1.5[c]	6	6	1-10

[a] Animals received single doses unless otherwise stated.

[b] Four received 12 doses and 4 received 24 doses on successive days.

[c] These animals were mothers of litters mentioned in Table 14 and received 30-120 doses during gestation, lactation, or both.

were treated with a 0.5-1.2 ml solution of MSG as above (see Table 15 for dosages).

The effects as shown by light microscopy were:

(1) In neonatal mice receiving 4-8 mg/g in single doses, there was necrotic damage to the ganglion cells, inner fiber layer, and some of the bipolar cells within a few hours after injection; the visual layer was unaffected. In very young animals (2 days) damage was more extensive.

(2) No such effects on the retina were observed at birth or afterward when the mother was treated during gestation and lactation.

(3) In adult mice only a partial lesion was produced by single doses of 4-8 mg/kg, i.e., the periphery of the retina was more affected than the central area. No mice receiving a dose of 1.0-1.5 mg/g daily for long periods (time not stated) were affected (see Table 16). Only 1 of 4 mice receiving 3-4 mg/kg for 12 doses showed a lesion, but all given 24 doses were affected.

Of the several other substances tested (glutamine, epinephrine, aspartate, ammonium carbonate) only sodium L-aspartate (1.27 mg/g BW) produced a similar lesion when injected s.c. in very young mice.

2. The observations of Lucas and Newhouse were confirmed in 1960 by Potts et al. (5878). They injected a minimum of 17 Webster Swiss strain neonatal albino mice (sex not stated) intraperitoneally (i.p.) with one MSG dose daily according to the dosage schedule shown in Table 17.

Controls showed no alteration in the normal electroretinogram (ERG). However, only a negative a-wave could be recorded in the ERG up to 3 months following treatment. The loss of the b-wave indicated that the inner retinal layers had not formed and thus the retinas consisted only of receptor cells.

Table 16. Results of Single Doses of Sodium L-Glutamate in Adult Mice (4487)

Dosage (mg/g)	Not affected	Animals, No. Severity of lesion[a]		
		+	++	+++
4	1	2	0	0
6	2	3	1	1
8	0	0	3	5

[a] +, the slightest detectable lesions; ++, lesions confined to the periphery; +++, lesion involving area centralis.

Table 17. Mouse Glutamate Dosage (5878)

Age (days)	Dose (mg/kg BW)
2	2.2
3	2.5
4	2.8
5	3.2
6	3.4
7	3.6
8	3.8
9	4.0
10	4.2
11	4.4
12	4.6
13	4.8
14	5.0
15	5.2
16	5.4
17	5.6
18	5.8

This finding led the authors to hypothesize that the development of the inner retinal layers had been repressed by giving large doses of MSG during the developmental period for these layers. They further hypothesized that the high glutamate levels given repressed the activity of glutaminase (which converts glutamine to glutamate). Thus, formation of the inner retinal layers would be inhibited if this enzyme were essential to their development. Two reports of the experimental testing of this hypothesis on rats are given on pp. 56-58.

3. In 1967 Cohen (1432), using electronmicroscopy, studied the retinal effect of MSG on 35 neonatal Swiss albino mice and 10 neonatal C3H/HeJ mice (as well as control litters) two months and one year following treatment. First the newborn mice were injected s.c. with MSG for 18 days, starting with the second postnatal day, as per Potts et al. (5878). Then it was found that the same pattern of degeneration took place when only 10 injections were given on the first to tenth postnatal days. Controls injected with water or saline showed no discernible effect.

The eyes of the experimental animals appeared smaller two months following MSG treatment and the lenses averaged 65% of the weight of the controls. At the same time, a reduction was noted in myelinated axons of the optic nerve, from about 25,000 to less than 225, and the inner nuclear layer of the Swiss mouse retinas was reduced from 5 to 7 nuclei deep to 1-2. The author concluded that the effect of MSG on the retina appeared to be partial general destruction of the inner retinal layers with little immediate effect on the receptor cells.

4. Using a single s.c. injection of MSG (4 mg/g), Olney (5481) in 1969 produced acute degenerative retinal lesions in mice. Three litters of 9- to 10-day-old Swiss albino mice were injected once s.c. with an 0.2-ml solution of

MSG in distilled water, a dose level of 4 mg/g BW. The commercial MSG used did not show the presence of impurities when analyzed by thin-layer chromatography. Animals were sacrificed between 30 minutes and 48 hours; their retinas were fixed in situ and then dissected for light and electron micrography. Early signs of an acute degenerative process were documented at three hours after injection, and by 48 hours the degenerated neurons "were almost entirely eliminated, and an overall reduction of retinal thickness to approximately 65 percent of normal was evident." Other observations were:

(1) MSG treatment had no primary effect on photoreceptor cells and the majority of cells in the outer two or three tiers of the inner nuclear layer.

(2) The same treatment given to 1 to 3-day-old mice tended to produce a milder lesion limited to the ganglion cells.

(3) Daily injections from the first to tenth days (as per Cohen, 1432) also resulted in lesions approximately the same size as those produced by a single injection (4 mg/g) given on the tenth postnatal day.

(4) Lesions that increased in severity up to the tenth day of age were produced with a single injection as above.

(5) After the tenth day, resistance appears to develop and even with lethal doses it was difficult to produce lesions.

(6) Untreated control littermates had histologically normal retinas.

B. Rats

1. After the experiment on mice reported in 1960 by Potts et al. (5878, see pp. 52, 54, 55), they had hypothesized that the tissue damage was associated with suppression of retinal glutaminase activity. (Of the

glutaminases I and II that liberate ammonia from glutamine, glutaminase I is phosphate-dependent and has a lower optimum pH, whereas glutaminase II activity involves two enzymes, a transaminase and a deaminase.) In 1962 Freedman and Potts (2235) reported some studies in rats.

Albino rats (Sprague-Dawley strain) were injected i.p. with MSG according to the dose schedule used in their previous experiment (Section II, A, 2) from the second through the twelfth days of age. They found that:

(1) The rat retinas showed the same loss of inner retinal layers and of the β-wave as reported for MSG-treated mice (Section II, A, 2).

(2) Glutaminase I activities in brains and livers were not significantly altered by treatment.

(3) The activity of glutaminase I in the control retinas was nearly twice that in the treated rat retinas, and the control rat retinas contained almost twice as much of this enzyme per unit weight as those of the MSG-treated rats.

(4) There was no indication of changes in the activity of the other three enzymes metabolizing glutamate or glutamine; glutaminase II, glutamosynthetase, and glutamotransferase.

The authors concluded that this experiment supported their hypothesis (pp. 52, 54, 55) that the effect of MSG on the retina could be explained by endproduct feedback inhibition of glutaminase I synthesis, emphasizing that such inhibition was postulated to be lethal or to prevent cell development. They commented that competitive inhibition, as described by Krebs, could also be consistent with their results and their hypothesis.

2. Freedman and Potts (2236) next employed isotopically labeled MSG to elucidate some details of the mechanisms of action of glutamate in sensitive

retinas. Their specific objectives were:

(1) To assay retinal enzyme activity.

(2) To analyze the pattern and quantity of tissue uptake of MSG in newborn rats of various ages. For this study 3.2 mg/g BW of isotopically labeled MSG was injected i.p. in 5-day-old rats (number, sex, and strain not given).

(3) To elucidate the type of inhibition of glutaminase I.

Their findings, reported in 1963, were that:

(1) Glutaminase I specific activity was decreased 48% in the retinas of treated animals as compared to controls, a revised estimate owing to improved accuracy. Glutaminase II activity was present in retinas and was unaltered by treatment. A 40% increase in glutamic oxaloacetic transaminase specific activity was found in the retinas of the treated rats as compared to the controls.

(2) On the fifth postnatal day there was a high uptake of isotopically labeled glutamate from plasma by brain and retina, which declined on the tenth and again on the twelfth postnatal day.

(3) However, peak tissue levels of glutamate, observed for 3.5 hours, 1.5-5 hours after MSG on day 5, corresponded to only 10% inhibition of glutaminase I on a curve for its inhibition by glutamate <u>in vitro</u>.

The authors concluded that endproduct-inhibition of new enzyme synthesis was more likely than competitive inhibition of enzyme activity. However, Olney in his 1969 paper (5481, see pp. 55-56) suggested that their findings could also be explained by development of a retinal barrier to uptake of glutamate after the fifth postnatal day, such as would not interfere with blood supply to the inner retina.

3. Hansson (2804) in 1970 found rat retinal nerve cells to be more severely damaged than those in mice. In the rats only a few small nerve cells remained after glutamate treatment, as compared to thousands in mice (citing 4487,1432, 2235,5481). An unstated number of albino Sprague-Dawley suckling rats of both sexes were injected i.p. with MSG in increasing amounts according to Lucas and Newhouse (see pp. 49-52), for 14 days from the second day after birth. Controls were similarly injected with saline. Additional observations were made in some animals two to six months after treatment:

(1) MSG-treated rats were shorter and fatter than their control littermates. [Compare observations on pp. 66-69 (5488) and p. 82 (6053)].

(2) The eyes were smaller and the lenses lighter in the MSG-treated rats than in the controls.

(3) No obvious behavioral differences were observed.

(4) There was almost complete degeneration of retinal nerve cells, including the processes and synaptic connections in the MSG-treated ratlings.

The author described his investigation by transmission and scanning electronmicroscopy of the surface structures, and the reactive changes observed in the neuroglial cells and the blood vessels in the rat retinas several months after MSG treatment.

4. In a related study in 1970 Hansson (2805) described the ultrastructure of MSG-treated ratling retinas. The conditions were similar to the previously described experiment (2804), with two exceptions:

(1) The controls were injected with the same amount of water used to dissolve the MSG.

(2) While most of the animals were treated as described by Lucas and Newhouse (4487, see pp. 49-52), retinal damage of a

similar extent was produced with a single MSG injection (dose not stated).

A total of 31 litters (80 rats) were used. One-third to one-half of the test animals suffered localized or generalized seizures and only 48 survived. As in some of the previously described retinal studies, the nerve cells in the ganglion cell layer and inner nuclear layer were in some cases almost totally destroyed while the photoreceptor cells and epithelial cells appeared relatively unchanged. Additional observations included disappearance of most blood vessels in the severely damaged retinas, and appearance of "special" glial cells.

The times of appearance of the retinal damage and the rates of development of the early stages were not reported in either of Hansson's experiments (2804, 2805).

C. Rabbits

1. In the previously described experiments with mice and rats (see pp. 49-60), adult animals appeared to be resistant to MSG-induced retinal damage.

In 1964, Hamatsu (2770) demonstrated for the first time that such retinal degeneration could be produced in the adult rabbit. Twenty-one rabbits (ca. 2.0/kg/BW) were injected i.p. once a day for 16 consecutive days with a 25% aqueous solution of MSG in dosages of 2.0 g/kg (five rabbits); 1.0 g/kg (five rabbits); 0.5 g/kg (three rabbits); 0.25 g/kg (five rabbits); 0.1 g/kg (three rabbits). The animals were killed after 16 days.

The observations made were:

(1) At the 2.0 g/kg level there were marked histological changes evidenced by regressive degeneration in the pigment epithelial layer,

the inner and outer segment of the visual cell, the internal and external molecular layer, and the ganglion cell layer. Periodic acid Schiff (PAS) and alkaline phosphatase (ALPase) reactions were also compared in the various layers and found to vary from the normal. The most notable observation at all dosage levels was that the ALPase reaction was stronger than normal in the ganglion cell layer. The amplitudes of both a- and b-waves of the electroretinogram (ERG) decreased markedly until the 16th day when they were half of those before MSG administration, indicating considerable retinal lesions.

(2) At lower doses of 1.0 g/kg and 0.5 g/kg, the locations of the lesions were similar to those at 2.0 g/kg but the histological changes were smaller. Decreases in the amplitudes of the a- and b-waves continued to indicate lesion development.

(3) At the lowest dose levels, 0.25 g/kg and 0.1 g/kg, no histological changes were observed. There was a variation in a- and b-wave amplitudes, however, of which the interpretation was uncertain.

(4) MSG selectively damaged the inner retinal layers in the adult rabbit.

2. Kobayashi (3862) in 1970 studied the changes in the retina of the adult rabbit with an electronmicroscope when a small dose of MSG (0.1 g/kg in 25% solution) was intravenously (i.v.) injected (auricular vein) for 50 consecutive days. The nine adult albino rabbits (weighing more than 2.0 kg) which were used in the experiment were killed on the 10th day (three rabbits), on the 30th day (three rabbits), and on the 50th day (three rabbits).

See Table 18 for changes observed in the synaptic vesicles over the duration of the experiment.

Table 18. Distribution of the Synaptic Vesicles in Outer Plexiform Layer (3862)

Subjects	Number of the vesicles in 0.5 μ^2	Diameter and percentage of the vesicle					
		21∿30 nm	31∿40 nm	41∿50 nm	51∿60 nm	61∿70 nm	71∿80 nm
Normal	29	-(%)	23(%)	72(%)	5(%)	-(%)	-(%)
10th day	32	-	4	76	20	-	-
30th day	25	-	34	52	14	-	-
50th day	12	12	73	10	5	-	-

Other changes observed by electronmicroscope were in the inner segment of the visual cells and the external plexiform layer; these were markedly affected, even though the ERG amplitudes were nearly normal.

III. Toxic Effects: Brain Damage, Neuroendocrine Disturbances, and Nephrotoxicity

Introduction

In August 1970 at the time of the completion and submission of the report of the Subcommittee on Safety and Suitability of MSG and Other Substances in Baby Foods, National Academy of Sciences/National Research Council (NAS NRC, 5226), the evidence for MSG-produced lesions in the hypothalamus of neonatal animals was limited and conflicting.

However, since then a number of findings of MSG-induced brain lesions have been reported for mice, rats, chicks, rabbits (unpublished, quoted in 6283), and monkeys. Other reported findings include obesity and neuroendocrine disturbances in mice and rats, and nephrotoxicity in chickens. These are outlined in this Section together with some studies on the mechanism of the brain damage observed.

Doses of MSG that have been reported to produce lesions in the hypothalamus are shown in Table 19. In some studies lower doses failed to produce such lesions, and in others (0307,5530,0032,1150,6095) the authors reported either failure to produce lesions or changes that they would not interpret as lesions. Doses for other effects are summarized in Table 20.

Table 19

Hypothalamic Lesions Reported with MSG

Route	Dose g/kg	No. of doses (once daily)	Animals	References
s.c.	0.5-4.0	1	mouse	5488
	3.0	1	mouse	0306
	2-10	1	mouse	0305
	2.4	1-5	mouse	4747
	0.5	5		
	1.0	1	mouse	5130
	5.0	1	mouse & fetus	5130
	0.25-4.0	20	rat & fetus	2047
	1-4	1	chick	6643
	2.7	1	monkey	5490
i.p.	2-10	1	mouse	0305
	4	1	chick	4025
oral	0.5 (52%) 0.75 (82%) 1.0 (100%)	1	mouse	5491, 5492
	0.5-2.0	1	mouse	5483
	1.0 (100%)	1	mouse	1053
	4.0	1	mouse	4327
	1.0	1	rat	1053
	1.0-2.0	1	monkey	5485

Table 20

Obesity, and Endocrine, Convulsive, or Kidney Disorders Reported with MSG

Route	Dose g/kg	No. of doses (once daily)	Animals	Observations	References
s.c.	2.2	15	rat	obesity	2804
	2.2-4.0	10	mouse	obesity	5488
	not stated	10	rat	obesity	3859
	2.2-4.0	1-5	mouse	obesity, endocr.	4747
	2.2-4.2	10	rat	gonads, endocr.	6052, 6053
	4.0	1	monkey	convulsions	5485
i.p.	2-4		rat	convulsions	5143
	4.4		rat	convulsions	0746
	0.5-20 mmol/kg		rat	convulsions	3436
	4	1	chick	abnormal EEG	4025
oral	5		rat	behavior	5884
	10% in diet		rat	behavior	7915
	0.3-2.6	in water 28 d	chick	gouty kidney	6714
	unknown	unknown	gouty humans	high blood glutamate	5586

A. Mice

1. The idea that MSG might cause neuronal damage in areas of the central nervous system (CNS) other than the inner retina, such as the hypothalamus, suggested itself to Olney (5488) from the observation that several months after MSG treatment in a retinal study, neonatal mice became obese (5488, footnote 10). In 1969 he reported the first observations of brain lesions in the arcuate nucleus of the hypothalamus of neonatal mice, following administration of MSG.

Ten litters of Swiss albino mice (total number and sex unstated) 2-9 days old were killed 1-48 hours after single s.c. injections of MSG from 0.5 to 4 mg/g BW. Within "a few hours," acute lesions were seen in the preoptic and arcuate nuclei of the hypothalamus, characterized by intracellular edema and neuronal necrosis. The ventromedial nucleus appeared unaffected. These lesions were found with every dose tested.

Doses of 5-7 mg/g BW given s.c. to adult mice also resulted in acute brain lesions. Neonatal C57BL/6 mice and albino rats also developed brain lesions after MSG treatment (dose not stated).

In a second study of longer range effects resulting from MSG treatment, 20 neonatal Swiss albino mice from five litters were injected s.c. from one to ten days following birth with MSG in graduated doses from 2.2 mg/g BW on the first day to 4.2 mg/g BW on the tenth day [dose schedule according to Cohen, (1432)]; 18 controls received no treatment.

Figures 1a and 1b present growth data for females and males respectively. In addition, food consumption data for all males studied are shown in Fig. 1b.

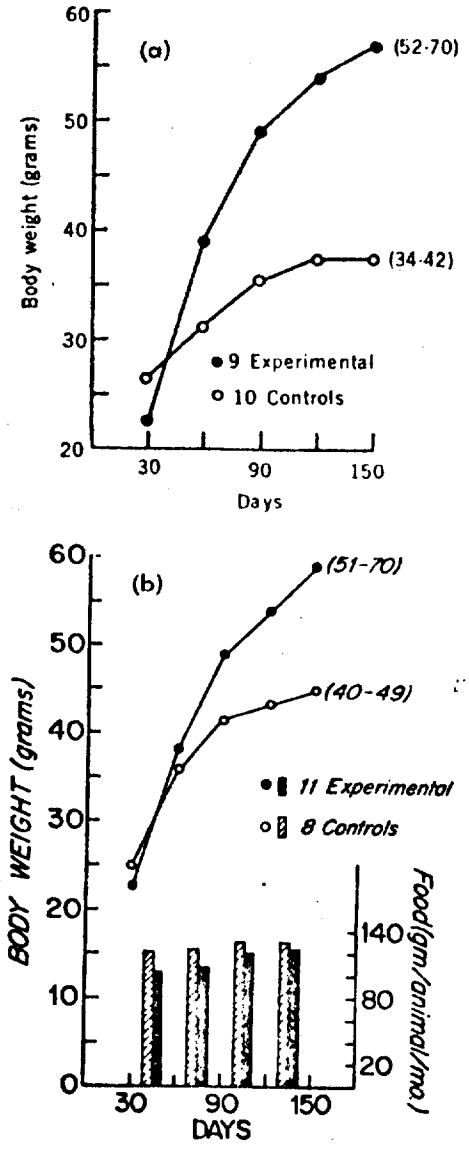


Fig. 1 (a) Composite growth records for experimental and control females from five litters of mice covering the first to fifth months of life. Weight ranges on the 150th day are in parentheses. (b) Composite growth records and data on food consumption for experimental and control males from five litters of mice covering the first to fifth months of life. Ranges of weight on the 150th day are in parentheses. (5488)

It was noted that:

(1) At the end of treatment, experimental animals appeared stunted and remained smaller than controls through weaning up to the 30th day.

(2) Thereafter, treated animals gained more weight than controls, experimental females exceeding both experimental males and control females.

(3) Despite excessive weight gains, treated animals were about 10% shorter in mean body length than controls, reflected in measurements of spinal and long bones.

(4) Treated females were sterile, males were fertile. This was confirmed by examination of the gonads at nine months. Ovaries contained about twice as many atretic follicles as the controls and the uteri appeared slender and attenuated, with thinner endometrium and small secretion-poor glands. Testes were indistinguishable from controls.

(5) At nine months there were "massive accumulations of adipose tissue in experimental animals compared with small to moderate amounts in controls". Treated animals had fatty livers.

(6) The anterior pituitaries of the adult animals treated in infancy were abnormally small even though examination of infant pituitaries one to 48 hours after treatment revealed no acute degenerative changes. The author interpreted this as an interference, "perhaps hypothalamic," in the development of the pituitary.

A single s.c. injection of MSG (3 mg/g BW) given at two days of age to ten Swiss albino mice resulted in a more gradual and less severe obesity. Treated animals averaged 16.9 g heavier than 13 control littermates at nine months.

The author concluded that appetite disturbance could not explain the obesity because no lesions were found in the ventromedial nuclei and (see Fig. 1b) the treated animals ate less than the controls.

However, in 1973 Gold (8360) reported a quantitative study of hypothalamic lesions produced by knife or electrocautery in rats, and concluded: "Lesions restricted to the ventromedial nucleus of the hypothalamus were neither necessary nor sufficient for, and did not contribute to, the production of hypothalamic obesity. Hypothalamic lesions and knife cuts that do produce obesity damage the nearby ventral noradrenergic bundle or its terminals."

2. Hypothalamic damage in infant mice following "relatively low oral doses" of MSG was first reported by Olney and Ho (5492) in 1970. In this experiment other substances were also administered as shown in Table 21.

Single doses of a 10% aqueous solution of MSG were given by intubation to 75 Webster Swiss albino mice 10-12 days old, at one of the five following dose levels: 0.25, 0.5, 0.75, 1.0, and 2.0 g/kg BW. Ten controls were intubated without treatment. Animals were killed "about" five hours later, by a perfusion-fixation technique that prepared the tissues for either light or electron microscopy.

As shown in Table 21:

(1) No lesions were produced at the lowest dose, 0.25 g/kg BW.

(2) The next dose, 0.5 g/kg, produced lesions in 12 of 23 animals treated (52%).

(3) At 0.75 g/kg 13 of the 16 test animals (81%) had lesions.

(4) All 19 of the test animals at 1.00 g/kg and all seven of the test animals at 2.00 g/kg developed arcuate lesions.

Forty-six mice of the same strain were similarly given other test compounds (see Table 21) in single doses of 3 g/kg BW. Findings were:

(1) No hypothalamic pathology with either sodium chloride or sodium glutarate.

Table 21 (5492)

Test compound	Dose (g/kg)	Number treated	Number affected	Necrotic hypothalamic neurons
Intubated, no treatment	---	10	0	0
MSG	0.25	10	0	0
MSG	0.50	23	12	7
MSG	0.75	16	13	13
MSG	1.00	19	19	25
MSG	2.00	7	7	40
L-Glutamic acid	1.00	4	4	23
Monosodium L-aspartate	1.00	4	4	26
L-Glutamate L-aspartate	0.50/0.50	8	8	27
Monosodium-glutarate	3.00	4	0	0
NaCl	3.00	4	0	0
L-Glycine	3.00	2	0	0
L-Serine	3.00	2	0	0
L-Alanine	3.00	2	0	0
L-Leucine	3.00	2	0	0
DL-Methionine	3.00	2	0	0
L-Phenylalanine	3.00	2	0	0
L-Proline	3.00	2	0	0
L-Lysine	3.00	2	0	0
L-Arginine	3.00	2	0	0
L-Cysteine	3.00	4	4	57

Each of the listed compounds was given in 10% aqueous solution except L-glutamic acid, L-leucine, DL-methionine, and L-phenylalanine which were given in 25% aqueous solution because of the poor solubility in water. Because a large volume of fluid was needed to deliver high doses of L-leucine, DL-methionine and L-phenylalanine, only half the dose was given orally and the remainder subcutaneously. All of the other compounds were given orally. Sources of L-glutamic acid and MSG were purity checked by thin layer chromatography. Figures in the necrotic hypothalamic neuron column represent averages for each dose level.

(2) L-Glutamic acid at 1.0 g/kg BW destroyed approximately the same number of hypothalamic neurons as did an equivalent dose of MSG.

(3) Of the other amino acids tested only cysteine and monosodium L-aspartate (MSA) produced findings, i.e., retinal and hypothalamic lesions in all treated animals similar to those produced by MSG.

(4) The lesions observed after 0.5 g/kg of MSG was given together with 0.5 g/kg of MSA (Table 21) suggested the possibility of an additive effect.

The authors commented that glutamate, aspartate, and cysteine had been classed as "neuroexcitatory" amino acids because of their special ability to depolarize nerve membranes. Whether this was relevent to their observations was a question for further study (5492).

3. In 1971 Olney et al. (5486) compared the cytotoxic potencies and specificities of 24 compounds, related structurally to MSG, on the hypothalami and retinas of 250 10-day-old Webster Swiss albino mice. All the compounds were found to fit into four groups: (1) Equipotent with L-MSG in necrosing neurons. (2) More potent. (3) Affecting non-neural CNS components (glial, ependymal, Muller cells). (4) No effects. Relationships were observed, between structure and specificity, that suggested similar mechanisms for the neuroexcitatory and neurotoxic properties of the simple amino acids, glutamic, aspartic, and cysteic. See the paper itself for details.

4. Arees and Mayer (0307) reported in 1970 that they were consistently able to produce lesions in the brains of Swiss albino mice (Charles River; CD-1) with single doses of MSG administered either s.c. or i.p., but that the subsequent lesion sites were apparently smaller than Olney's (5486, see above) and at three hours involved largely microglial cells while

sparing hypothalamic neurons. At 24 hours glial cell damage was more advanced, but no further advance was seen at 72 hours. The authors commented that MSG might or might not have an affinity for glial cells, and suggested that Olney's more extensive findings (5487,5488,5490 cited) might be due to poor fixation resulting from the reported vascular degeneration, since both laboratories were using similar fixation techniques.

However, the same authors (0306 and 0305 below) later found neuronal degeneration in infant mouse brains by several histological methods after s.c. or i.p. injections of MSG.

5. In 1971 Arees et al. (0306) reported MSG-induced damage to neurons of the lateral geniculate nuclei of day-old mice. Thirty-seven were injected s.c. with single doses of MSG 3 mg/g BW and were killed 30 minutes to seven days later. Degeneration of the optic pathway including fiber and terminal necrosis in the area of the subiculum was observed at 24 hours. The authors cautioned against premature extrapolation from mice to men.

6. In 1972 Arees and Mayer (0305) injected infant and adult mice s.c. or i.p. with 2-10 mg/g MSG/BW and killed them 30 minutes to two weeks later. Hypothalamic microglial degeneration was first seen at three hours and was maximal at 24 hours, when distal axonal degeneration was also seen. Terminal degeneration in subiculum and lateral geniculate nuclei was found in 7-day-old mice 24 hours after treatment, and the authors concluded that "brain lesions are more extensive than at first suspected."

7. A "more complete morphological description" than had appeared previously of hypothalamic damage induced by the acidic amino acids was given by Olney in 1971 (5483). He reported data from a study performed during the previous year on 300-400 infant animals, mostly mice, and involving MSG and related test

compounds. All animals were killed by perfusion-fixation, as before (5492, see p. 69) but fully described again in this report (5483).

The MSG-treated mice showed a "clearly demarcated lesion involving the entire arcuate nucleus but not extending into other adjacent hypothalamic regions", while the brains of untreated and NaCl-treated controls showed no pathological changes. In the treated mice, glial and ependymal cells were damaged reversibly, but arcuate neurons were selectively destroyed. Phagocytosis of debris was almost complete within 48 hours, "so that few traces, other than a reduced population of neurons, were found in brains examined more than two to four days following treatment."

The author argued that this selectivity of neuronal destruction could account for later neuroendocrine disturbances (e.g., obesity) seen in MSG-treated animals but could not itself be explained as secondary either to ischemia or to glial cell damage. On the other hand there was no evidence for the attractive theory that the blood brain barrier might be regionally permeable to MSG. Since glutamic acid was a common CNS metabolite, a metabolic explanation seemed preferable, and the author again pointed to the neuroexcitatory properties of the same group of amino acids that had been found neurotoxic.

8. Oser et al. in 1971 (5530) reported a comparative study on the potential toxicity of MSG from a food safety standpoint. Single doses of 1 g/kg BW of MSG, monopotassium glutamate, sodium chloride, or sodium glutamate in aqueous solutions, or distilled water were administered orally or s.c. to 3-day-old animals, (C57BL/65 mice, FDRL-Wistar-derived rats, and beagles) in groups of five, sex unstated, for each experimental treatment. Controls were also used for each test. In addition, a second series of tests was done at 12 days of age for the rats and mice and at 35 days for the beagles. The animals were killed 24 hours after dosage.

Mice and rats were autopsied after rapid anesthetization with ether; their brains were not perfused but were dissected and placed in fixative (formalin or glutaraldehyde). Dog brains were perfused with 3% glutaraldehyde "for preservation." Methods of killing were not reported. Tissues were stored in phosphate buffer and were stained with H and E for light microscopy.

The authors found no significant differences between test and control groups. Scattered observations of neuronolysis and neurophagia were neither localized nor related to treatments. They were interpreted as related to the speed of growth of neonatal brain. The authors declined comment on the differences between their observations and Olney's.

9. Burde et al. (1053) undertook a blind study to resolve the discrepancies between the findings of Olney (5488,5492,5490 cited) and those of Oser et al. (5530 cited), Adamo and Ratner (0050 cited, see p. 80) and Arees and Mayer (0305 cited). Using Olney's techniques (Table 22), and independent examiners, they reported arcuate lesions in all infant mice treated orally with MSG 1 mg/g and in none of their controls. Their rat findings are given on p. 80.

The authors commented that Oser et al. (5530) had not examined their animals until 24 hours after a minimally effective dose, and then had reported findings that, on detailed examination, were "entirely compatible" with late-stage Olney-type lesions. They (1053) expressed puzzlement that Arees and Mayer (0307) had found glial but not neuronal involvement (but see 0306,0305 on p. 72), and concluded that "exact duplication of experimental protocols" was required for valid comparisons.

Olney et al. (5486, see p. 71) pointed to the later findings of Arees et al. (0306 and personal communication) and to the use by Oser et al. (5530 above) of "relatively unrefined" tissue preparations. They also commented

Table 22. Treatment Schedule and Fixation (1053)

Animal	Method of administration	Treatment		Perfusion method
		MSG	NACl/saline	
Mouse	Oral	9(1 mg/g)	9	Olney
Rat	Oral	5(4 mg/g)	4	Olney
Rat	Oral	4(4 mg/g)	4	Adamo and Ratner
Rat	Subcutaneous	6(4 mg/g)	4	Adamo and Ratner
Rat	Subcutaneous	2(4 mg/g) 2(2 mg/g)	2	Olney

that although both Oser et al. (5530) and Burde et al. (1053) had given MSG 1 mg/g to mice, Burde et al. had intubated the dose and killed the animals five hours later [not shown in 1053] whereas Oser et al. had failed to intubate and had waited 24 hours.

10. In 1971 Abraham et al. (0032) reported arcuate lesions in 42% of 5-7-day old Swiss Albino and C57BL mice given MSG 1 g/kg s.c. and in 60% of those given 4 g/kg s.c. or by tube. The mice were decapitated after 1, 3, 12 or 24 hours, and their hypothalami were fixed without perfusion, partly in formalin and partly in glutaraldehyde. In the affected mice neuronolysis was associated with s.c. doses, and oral doses with glial cell infiltration. The arcuate neuronolysis was advanced by three hours, and by 12-24 hours few traces were left. A "dramatic" but unexplained increase of myeloid bodies, interpreted as lysosomes, was observed in neuronal, glial and ependymal cells of affected hypothalami, along with a loss of acid phosphatase activity.

11. Lemkey-Johnston and Reynolds (4327) summarized, in 1972, their observations of the extent of brain lesions in neonatal mice (number, strain, and sex not stated) following oral 4 g/kg doses of MSG. At 20 minutes arcuate necrosis was seen; at 30 minutes arcuate and preoptic neurons, and at 60 minutes epithalamus and habenar nuclei, were involved. At two hours the tectum was damaged "extensively" in some mice, and at 24 hours arcuate neurons were replaced by phagocytes. Serial sections at three hours revealed additional damage to exterior cortex, olfactory bulbs, claustrum, caudate putamen, dorsal lateral thalamus, hippocampus, vestibular nuclei, and both superior and inferior colliculi. The authors commented that these additional regions were all proximal to cerebrospinal fluid.

12. In 1970 obesity in MSG-treated mice was reported by Matsuyan (4747) in Japan. Infant ICR mice (both sexes, number not given) were given MSG s.c.

in three different dosages: 0.5 mg/g (every other day for five treatments); 2 mg/g (one to five treatments); and 4 mg/g (only one treatment).

Matsuyan observed the following:

(1) Brain tissue of MSG-treated mice studied two hours after treatment with 2 mg/g and 4 mg/g showed necrosis of neurons in the third ventricle of the hypothalamus.

(2) Obesity was seen in the mice given the 2 mg/g and 4 mg/g doses.

(3) No obesity was produced with 0.5 mg/g dose.

(4) Obese mice and controls had the same growth pattern until eight weeks of age. Thereafter experimental animals gained at a rapid rate, even after 50 weeks of age.

(5) Massive amounts of abdominal fat were revealed by autopsy.

(6) Livers were heavier than normal, showing fatty degeneration.

(7) Estrous cycles and sperm motility of obese mice were not affected.

The author also noted that puberty was delayed in female rats treated with MSG (no further information on this study was given in this paper).

13. Murakami and Inouye in 1971 (5130) reported confirmation of Olney's findings (5488) that MSG administered s.c. destroyed neurons in the infant mouse hypothalamus. They also produced brain lesions in mouse fetuses after the treatment of pregnant mice late in gestation with MSG or MSA.

In a pilot experiment 13 neonatal CF#1JCL mice were injected s.c. with MSG 1 mg/g, and untreated and saline controls were prepared. Brains were removed 1, 3, 6 and 24 hours later and were fixed without perfusion. Light microscopy revealed neural necrosis at three or six hours localized symmetrically in the arcuate nuclei, median hippocampus and medial habenular nuclei. Ependymal cells were affected in the lower part of the third ventricle.

Next, 26 mice were given 5 mg/g s.c. on day 17 or 18 of pregnancy. One litter was examined at one week and another at three weeks of age, and findings were negative. The remainder were removed 3, 6 or 24 hours after treatment, and the findings were:

(1) In the fetuses treated on the 17th day, of 24 randomly selected animals from 8 litters, 7 showed acute lesions located in the ventromedial nucleus of the hypothalamus.

(2) In the fetuses treated on the 18th day, of 27 animals randomly selected from 10 litters, 6 showed brain lesions now located in the arcuate nucleus of the hypothalamus.

(3) Similar lesions were seen in the mothers killed at 3 hours.

(4) No lesions were seen in 4 "cases" examined at 24 hours.

The authors reported, without details, that MSA given on day 18 of pregnancy produced pyknosis of the lower hypothalamus of the fetuses seen 3 or 6 but not 24 hours after treatment. They compared the lesions to those produced by gold thioglucose.

14. Van Gelder (7648) offered adult Sprague-Dawley ARS Ha/ICR mice a constant diet supplemented with 0.5% L-glutamic acid, glycine or γ-aminobutyric acid (GABA). The L-glutamate concentration was higher than reported in the Section on Consumer Exposure (2538,3440, see pp. 200-201). These diets were offered during breeding, pregnancy, and lactation, and to the subsequent offspring.

The L-glutamate-treated offspring were of the same size as control offspring at birth, but at 60 days treated offspring of both sexes were 20% smaller. Skull size and brain weight of females were normal, but that of males corresponded with their smaller size. All offspring were fertile, and brain examinations revealed "no obvious abnormalities."

However, the glycine and the GABA treated offspring had, at 60 days, significantly larger-than-normal skull sizes and brain weights.

The author tentatively commented that the glycine effects could be explained by enhancement of its incorporation into proteins, but that the GABA and glutamate effects could not; he surmized an assocation with the neuron-depressive property of glycine and GABA and the excitatory property of L-glutamate, perhaps with regard to CNS amino acid metabolism.

15. In 1971 Creasey and Malawista (1547) hypothesized that glutamate might inhibit transport of other natural metabolites, e.g., glucose, which was critical for brain function. They injected adult 30 g Swiss CD-1 mice with MSG 0.3 g/kg i.p. followed after 15 minutes with glucose-2-C^{14} 1 mC (0.62 mC/mmole) i.p. Controls received isotonic saline instead of MSG. Groups of six mice were killed at 10 minute intervals for one hour; heart-blood and brains were counted. MSG did not alter the counts in heart blood but significantly depressed brain counts up to 35.5% between 10 and 20 minutes after the glucose injection. MSG 0.6 g/kg increased the depression to 64.1%, indicating dose-dependence (1547).

The authors commented that satiety mechanisms, elevated uptakes of labeled glucose, toxic effects of gold thioglucose, and sensitivity to MSG, were all known to be localized within the hypothalamus; further, that they had depressed brain glucose uptake with less MSG than had been given by other workers who had demonstrated brain and retinal damage in mice, though not less than had provoked symptoms in sensitive humans (1547, and see pp. 108-123). Finally they noted that human plasma levels of glutamate were little more than one-fifth that of the mouse, and they suggested interference with brain glucose uptake as a "plausible" mechanism for MSG toxicity. [But see p. 184]

B. Rats.

1. Adamo and Ratner (0050) reported in 1970 that they were unable to duplicate Olney's findings (5488) with rats.

Wistar rats of both sexes, 3-4 days old, received single s.c. injections of MSG 4 g/kg in 0.1 ml, or saline. Some were killed after three hours by perfusion-fixation, and others were reared under controlled conditions, six males for 68 days and eight females for 88 days.

After light microscopy of preoptic and arcuate areas of the three-hour brains showed no damage, these areas from the treated adults were examined by electronmicroscope, and "there was no indication that such damage had ever occurred."

Females reared after MSG treatment cycled normally and produced normal litters. Though their ovaries weighed less than those of the controls, they were histologically similar. Weights of uteri, testes, seminal vesicles, and prostates were not significantly different from controls.

The authors attributed their negative findings possibly to variation in species susceptibility.

2. In a footnote, Arees and Mayer (0305) reported results which they stated were contrary to those of Adamo and Ratner (0050). Adult albino rats injected with 5 mg/g of MSG developed lesions in the arcuate nucleus of the hypothalamus.

3. In 1971 Burde et al. (1053) undertook a blind study to resolve these discrepancies, using mice (see p. 74) and Wistar rats. Treatments and fixation procedures both of Olney and of Adamo and Ratner were followed (Table 22), and tissues went to independent examiners. Necrosis of arcuate neurons was reported in all test animals but not in controls.

The authors commented that Adamo and Ratner's (0050) negative findings in the ultrastructure of adult rat brains were "entirely consistent" with Olney's (5483) finding of apparently normal residual cytology except for a smaller population of arcuate neurons. They stated that they could not explain Adamo and Ratner's negative three-hour findings. They added that reproductive dysfunction had not been reported after single doses of MSG during infancy but only after consecutive doses.

4. Everly (2047) administered MSG s.c. to several strains of rat fetuses (from day 16 to birth) and infant rats (days 1-21) at doses ranging from 0.25 to 4.0 mg/g BW. The brains were examined by light microscopy at 30 minutes, and one, two, and three hours after injection. Lesions were observed in the cerebral cortex, hippocampus, thalamus, and hypothalamus. This report (2047) is a brief abstract with few details.

5. Endocrine defects in rats given MSG in infancy were reported in 1970 by Redding and Schally (6052). Neonatal rats (sex, strain, and number not stated) were injected s.c. over ten days in increasing doses (2.2-4.2 mg/g BW) and autopsied on the 40th day. In the treated rats:

(1) Body weight and length in both sexes were less than in controls.

(2) Anterior pituitaries were significantly lighter, relatively and absolutely, and pituitary levels of thyroid stimulating and other tropic hormones were depressed.

(3) Ovaries and testes were atrophied with, respectively, 68% and 65% decreases in weight.

(4) Adrenals and thyroids of both sexes were significantly lighter.

The authors concluded that inhibition of endocrine functions by MSG could result from an effect on the hypothalamus and/or a higher CNS center.

6. Redding et al. (6053) reported endocrine defects after neonatal Sprague-Dawley rats (approximately 100 rats of both sexes) were injected with MSG s.c. from one to ten days following birth, in graduated doses from 2.2 mg/g BW on the first day to 4.2 mg/g on the tenth day, according to Potts et al. (5878). Controls received saline in adjusted volumes.

Rats from each group were separated according to sex and treatment and were sacrificed at 40 and 110 days of age. In the MSG-treated rats (see Tables in original paper for details):

(1) Body weights (BW) and nasoanal lengths were reduced significantly at 40 days, and lengths were 10-12% less at 110 days. Significantly more fat was accumulated. Food consumption of most rats was significantly reduced.

(2) Thyroid and adrenal weights of both sexes were significantly lower at 40 and 110 days, but the decreases relative to BW were marginal.

(3) Gonad weights were likewise lower; however, the testes appeared normal while the ovaries appeared atrophied, with many atretic follicles. Fertility of either sex was not reported.

(4) Anterior pituitaries of both sexes were significantly smaller than controls; they were lighter by over 50% at 40 days and remained unchanged. They contained less luteinizing hormone and growth hormone (by 71-84%), but not less thyroid stimulating hormone at 40 days. Follicle stimulating hormone was not measured.

The authors commented that their rats showed greater gonadal effects than did Olney's mice (5488 cited), and that their results could be explained on the basis of hypothalamic lesions, since Olney had found such lesions in rats as well as mice (personal communication cited). However, the authors explicitly did not rule out "direct toxic effects of MSG on the endocrine glands themselves."

7. In 1970 Knittle and Ginsberg-Fellner (3859) studied the mechanism of MSG obesity in the rat. MSG (amount not stated) was administered s.c. to newborn male Sprague-Dawley rats (number not stated) daily from days 1 to 10. Controls were untreated littermates.

In the treated vs. control rats:

(1) The epidydimal fat pads were significantly heavier (3.4 g vs. 1.8 g) owing to an increase in cell size (1.7 µg/cell vs. 0.2 µg/cell). There were significantly fewer adipocytes per pad (1.8×10^6 vs. 3.8×10^6).

(2) These cells were less responsive to the lipolytic effect of epinephrine, and more responsive to the antilipolytic effect of insulin.

The authors concluded that MSG produced obesity through these hormonal effects on the fat content of adipocytes, while reducing the number of adipocytes.

8. In 1971 Mushahwar and Koeppe (5143) gave infant Holtzman rats 1-4 g/kg of MSG, monosodium D-glutamate, MSA, glycine, or equimolar NaCl, by i.p. or intragastric injection. They found:

(1) Higher MSG doses invariably increased brain glutamine, never brain glutamate. MSA or glycine also increased brain glutamine, and glycine increased its own concentration.

(2) Convulsions were produced by i.p. doses of 2-4 g/kg MSG or monosodium D-glutamate, or 4 g/kg MSA, or (seldom and only after 90 minutes) by intragastric MSG 4 g/kg. MSG 4 g/kg also produced brain-surface hemorrhages in 14- to 15-day-old rats. Glycine and NaCl were not convulsive (although recipients seemed lethargic).

The authors concluded that the convulsions were due to the amino acid anions, not to ammonia, stating that D-glutamate was not deaminated by the rat. [See also p. 194].

9. In 1971 Bhagavan et al. (0746) induced spastic tremors and seizures in 57 of 119 adult male Sprague-Dawley rats by i.p. injections of MSG 4.4 g/kg. They also studied the influence of pyridoxine, which is directly involved in the metabolism of L-glutamic acid (GA) and γ-aminobutyric acid (GABA) (0746). Symptoms and their frequency during two hours after MSG are shown in Table 23, and the authors also noted:

(1) Five to 20 minutes after injection 99% of the rats were somnolent.

(2) One hour later 52% were salivating and 31% had spastic tremors (mild to severe) followed by running about the cage and stereotyped biting. This was significantly more frequent in rats on pyridoxine-control diet pretreated with pyridoxine than pretreated with NaCl, and less frequent in rats on pyridoxine-deficient diet or chow pretreated with pyridoxine (see Table 23 for diets and pretreatments).

(3) Rats usually recovered from seizures in two to three minutes and resumed spastic tremors until the next seizure, but 29 of 119 died.

(4) An additional eight rats were injected with NaCl, and four with sodium DL-lactate, at 2 mmol/100 g BW, to check effects of Na and of tonicity; none developed MSG-type symptoms.

(5) Five of 11 rats given electroencephalograms (EEG) had seizures while being recorded, which appeared as typical high-amplitude spikes.

The authors commented that others had provoked similar seizures in rats and dogs by brain injections of L-glutamate or pyridoxal phosphate, which could upset the balance between GA and GABA, mediated in the case of pyridoxal probably by an enzyme system. An alternative mechanism was through electrolyte imbalance, and detailed studies were needed on this effect of L-glutamate. There was a suggestion that such doses of L-glutamate given i.p. might cross the blood-brain barrier (0746).

Table 23 (0746)

Incidence of Somnolence, Hypersalivation, Spastic Tremors, Seizures and Death in MSG[a] - treated Rats

Diet	Pretreatment	Somnolence		Hypersalivation		Spastic tremors		Seizures		Death[b]	
		N	%	N	%	N	%	N	%	N	%
Laboratory chow (21)	Pyridoxine HCl[c]	20	95	6	29	5	24	1	5	2	10
Laboratory chow (22)	0.9% NaCl	22	100	16	73	7	32	5	23	6	27
Pyridoxine-control (25)	Pyridoxine HCl	25	100	15	60	15	60	7	28	9	36
Pyridoxine-control (25)	0.9% NaCl	25	100	17	68	6	24	5	20	8	32
Pyridoxine-deficient (15)	Pyridoxine HCl	15	100	4	27	2	13	1	7	1	7
Pyridoxine-deficient (11)	0.9% NaCl	11	100	4	36	2	18	1	9	3	27
Total 119		118	99	62	52	37	31	20	17	29	24

Figures in parentheses indicate the number of animals.

[a] 2 mmol/100 g body weight, intraperitoneal.

[b] During 24 h after injection.

[c] 5 mg/100 g body weight.

10. In 1971 Weiss et al. (7915) tested MSG at 10% in diet, and also isocationic amounts of monopotassium glutamate (MKG), NaCl, and KCl, in a search for changes in learning ability, motor activity, blood pressure, and convulsive threshold.

They found that:

(1) All groups had similar intakes, and MSG alone produced significantly low weight gains, measured after 12 and 24 weeks.

(2) At 24 weeks tail-cuff blood pressures were significantly high in the MSG and NaCl rats but not in the others.

(3) Motor activity was unchanged at 24 weeks, but response to amphetamine was diminished in the MSG and NaCl rats.

(4) Avoidance lever pressing failed to develop in the MSG rats but was enhanced in the MKG and KCl rats, suggesting a positive effect of potassium.

11. In 1971 Semprini et al. (8353) studied the effect of daily administration of MSG, added to the diet at different levels in two successive generations of Sprague-Dawley rats bred at the authors' institute. The food consumption of the various animal lots on the various diets studied is shown in Table 24. The diets contained three different vitamin mixture levels. The effects of these various vitamin mixtures were studied with respect to MSG fed at 0.5%, 1%, and 2% respectively. The authors noted that they decided to adopt this plan after accidentally observing that tolerance to dietary additions of MSG might be reduced if a diet were inadequate in vitamins.

Three experiments were carried out:

(1) The reproductive performance of two generations of female rats.

(2) The growth rate of two generations of both male and female rats.

Table 24. Daily Consumption of Diet (8353)

	During the pregnancy			1st - 15th day			16th - 30th day		
	Diet (g pro die)	MSG (mg tot. pro die)	MSG (mg. kg P.c. pro die)	Diet (g pro die)	MSG (mg tot. pro die)	MSG (mg kg P.c. nurse — rat pro die)	Diet (g pro die)	MSG (mg tot. pro die)	MSG (mg kg P.c nurse — rest pro die
MATERNAL GENERATION				Vitamines 0,5 %					
Control	13,2	0	0	14,3	0	0	28,0	0	0
MSG 1 %	15,8	158,0	592 3	16,2	162,0	691,1	24,4	244,0	846,6
MSG 2 %	14,8	296,0	1104,7	18,5*	310,0	1646,2	32,4	648,0	2491,3
				Vitamines 1 %					
Control	16,7	0	0	23,7	0	0	23,6	0	0
MSG 1 %	13,5	135,0	568,3	21,3	213,0	773,5	33,9	393,0	1235,2
MSG 2 %	16,6	332,0	1246,5	21 1	422,0	1496,1	42,1**	842,0	2777 1
				Vitamines 2 %					
Control	18,2	0	0	24,1	0	0	44,5	0	0
MSG 1 %	15,5	155,0	558 6	25,4	254,0	977,9	46,5	465,0	14ee,3
MSG 2 %	18,1	362,0	1277,2	24,6	492,0	1779,3	48,3	966,0	3011,8
1st GENERATION				Vitamines 1 %					
Control	17 3	0	0	....			....		
MSG 1 %	17,5	174,8	528,1	24,8	248,1	802,6	42 9	429,3	1184,2
MSG 2 %	15 4	308,6	1036,5	23,4	468,0	1485,9	35,4	707,6	2143,2
				Vitamines 2 %					
Control	17,4	0	0	....			....		
MSG 1 %	15,8	157,7	626,9	23,2	239,6	966,2	33,3	332,9	1097 8
MSG 2 %	17,6	359,4	1422,5	23 5	470,2	1834,5	33,5	669,6	2097,1

* 0,05 > P < 0,01; ** significance limit.

(3) The effect on the cellularity of the central nervous system (CNS) of two generations of male and female rats, as measured by DNA and protein analyses of homogenized whole brains.

The reproductive performance is summarized in Table 25. The authors concluded that at each dose of MSG, the number of accomplished pregnancies was increased and the survival rate at weaning was higher in the second generation.

The authors reported no dietary influence either on growth during the neonatal period or on the weight reached in adult life.

The effects on whole-brain cellularity, in terms of DNA content, are shown in Tables 26-30:

(1) No differences were seen at 0.5% vitamins, "probably due to a lower basic growth" (Table 26).

(2) At 1% vitamins and at 2% vitamins the first generation (Tables 27 and 29) showed significantly lower DNA, RNA, and protein values. Thus, "the total figure of nuclei is smaller and the cell dimensions are larger in rats from mothers which have been fed a high MSG diet." These differences disappeared by day 15 and beyond.

(3) In the second generation (Tables 28 and 30) on 1% vitamins, the cell numbers at birth were back to control values but by day 15 were smaller in the higher MSG group; at weaning both MSG groups had fewer cells than their parents at weaning (no control group is reported for these measurements).

(4) In the second generation on 2% vitamins (Table 29 and 30), again without controls, cell numbers were smaller than in their parents at day 15 for both MSG groups (significantly so for the higher MSG group) and again at weaning.

Table 25. Reproductive Performance (8353)

	Pregnancy %			Mean size of nest at birth			Mean weight at birth			Breeding %			Mean weight at weaning		
	C	MSG 1	MSG 2	C	MSG 1	MSG 2	C	MSG 1	MSG 2	C	MSG 1	MSG 2	C	MSG 1	MSG 2
MATERNAL GENERATION															
Vitamins 0,5 % . .	66,6	83 3	83,3	9	9	9	5,45	5,86	5,88	21,0	16.7	40,7	56,70	78,50	60,70
Vitamins 1 % . .	83,3	83,3	100,0	9	8	9	5,60	5,64	5,93	74,3	70,6	80,9	95,02	102,90	89 90
Vitamins 2 % . .	83,3	100,0	100,0	9	9	10	5,74	5,53	5,70	75,0	92.7	90,3	111,90	97,00	98,58
1st GENERATION															
Vitamins 1 % . .	80,0	85 7	71,4	8	8	7	6,10	5 84	5,60	0	56,1	36 1		76,00	79,50
Vitamins 2 % . .	80,0	100.0	100,0	8	7	8	5,63	5,87	6,11	0	87,2	83,9		70,14	74,75

Table 26. Nervous Central System Cellularity (1st Generation) (8353)

Vitamins 0.5 %

	RNA mg/g tissue	DNA mg/g tissue	Protein mg/g tissue	Nucleus number (millions)	Cellular size (nucleus weight) mμg	RNA / nucleus mμg	Protein / cell mμg
AT BIRTH							
Control	3,15 ± 0,84	2,67 ± 0 76	32,30 ± 10,19	90,46	2,49	7,55	75,2
MSG 1 %	3,30 ± 0,80	2,67 ± 0,42	30,23 ± 5,82	100,06	2,35	7,38	70,8
MSG 2 %	3,37 ± 0,48	2,48 ± 0,18	32,55 ± 0,53	97,47	2,50	8,36	80,6
AT 15th DAY							
Control	2,77 ± 0,01	1,30 ± 0,13	58,47 ± 6,43	243,32	4,75	13,22	278,8
MSG 1 %	2,90 ± 0,72	1,33 ± 0,20	67,21 ± 25,26	240 73	4,71	13,42	320 1
MSG 2 %	3,62 ± 0,78	1,42 ± 0,14	66,25 ± 21,31	264,39	4,41	15,73	269,0
AT WEANING							
Control	3,24	1,43	76 43	346,73	4,34	14,09	332,2
MSG 1 %	3,05 ± 0,45	1,54 ± 0,09	70,71 ± 8,14	375,14	4,02	12,09	283,5
MSG 2 %	3 15 ± 0,29	1,52	74,92 ± 3,94	358,08	4,08	12,84	305,5

Table 27. Nervous Central System Cellularity (1st Generation) (8353)

Vitamins 1 %

	RNA mg g tissue	DNA mg g tissue	Protein mg. g tissue	Nucleus number (millions)	Cellular size (nucleus weight) mμg	RNA / nucleus mμg	Protein / cell mμg
AT BIRTH							
Control	4,06 ± 0,35	2,96 ± 0 39	28,45 ± 2,10	102,77	2,11	8,27	80,4
MSG 1 %	2,37 ± 0,25*	2,11 ± 0,15**	30,78 ± 2,64***	81,44	3,09	7,36	96,0
MSG 2 %	3,08 ± 0,39***	2,22 ± 0,39**	25,88 ± 3,93*	82,99	3,00	8,69	72,3
AT 15th DAY							
Control	2,75 ± 0,46	1,31 ± 0,29	53,50 ± 5,00	247,86	4,93	13,23	259,7
MSG 1 %	3,04 ± 0,53	1,45 ± 0,40	56,54 ± 7,60	271,69	4,55	13,57	254,7
MSG 2 %	3,35 ± 1,18	1,23 ± 0,27	50,80 ± 6,59	221,05	5,21	16,61	260,5
AT WEANING							
Control	2,86 ± 0,22	1,36 ± 0,16	64,00 ± 1,41	331,94	4,62	13,21	296,0
MSG 1 %	2,86 ± 0,26	1,54 ± 0,10	71,92 ± 12,36	397,87	4,02	11,51	288,4
MSG 2 %	2,64 ± 0,24	1,34 ± 0,12	62,44 ± 4,18	348,58	4,63	12,17	288,9

* P < 0,001; ** P < 0,05; *** P < 0,01

Table 28. Nervous Central System Cellularity (2nd Generation) (8353)

Vitamins 1 %

	RNA mg/g tissue	DNA mg/g tissue	Protein mg/g tissue	Nucleus number (millions)	Cellular size (nucleus weight) mμg	RNA / nucleus mμg	Protein / cell mμg
AT BIRTH							
Control	4,27 ± 0,11	2,60 ± 0,16	42,33 ± 6,37	97,91	2,39	10,22	101,5
MSG 1 %	4,48 ± 0,48***	2,78 ± 0,40**	35,92 ± 4,60*	101,50**	2,28**	9,74*	80,8
MSG 2 %	4,19 ± 0,23***	2,69 ± 0,17**	31,30 ± 2,87*	104,24***	2,31**	9,69*	72,3
AT 15th DAY							
Control	....	....	....	....	....	....	....
MSG 1 %	3,58 ± 0,26	1,49 ± 0,07	37,00 ± 5,15**	269,46	4,16	14,85	153,8
MSG 2 %	2,62 ± 0,89	1,16 ± 0,30	31,03 ± 7,55*	190,69	5,62	13,78	167,7
AT WEANING							
Control	....	.....	....	....	....	....	....
MSG 1 %	2,10 ± 0,49	1,17 ± 0,29	30,44 ± 5,32**	218,17*	5,54	11,21	163,5**
MSG 2 %	2,07 ± 0,56	1,34 ± 0,33	33,73 ± 7,34***	275,10	5,22	10,43	172,2**

The values of significance here reported are referred to the corrisponding groups of the 1st generation.
*** $P < 0,001$; ** $P < 0,01$; * $P < 0,05$; • significance limit.

Table 29. Nervous Central System Cellularity (1st Generation) (8353)

Vitamins 2 %

	RNA mg g tissue	DNA mg g tissue	Protein mg g tissue	Nucleus number (millions)	Cellular size (nucleus weight) mμg	RNA / nucleus mμg	Protein / cell mμg
AT BIRTH							
Control	4,07 ± 0,66	2,95 ± 0,36	27,37 ± 5,48	116,5	2,11	7,59	58,3
MSG 1 %	3 47 ± 0,45	2,83 ± 0,40	28,60 ± 6,68	99,24	2,22	7,70	62,7
MSG 2 %	3,19 ± 0,44**	2,77 ± 0,45	27,70 ± 5,01	92,93	2,30	7,21	63,3
AT 15th DAY							
Control	2,47 ± 0,48	1,55 ± 0,15	50 38 ± 7,99	301 85	4,01	9,83	200,2
MSG 1 %	3,33 ± 0 89	1,52 ± 0 16	57,77 ± 12,89	289,14	4,07	13,1	233,5
MSG 2 %	3,16 ± 0,30**	1,44 ± 0,08	51,32 ± 7,11	269,97	4,30	14,34	205,7
AT WEANING							
Control	2,77 ± 0,37	1,26 ± 2,55	65,45 ± 16,49	323,23	5,08	13,91	320,7
MSG 1 %	2,57 ± 0,26	1,38 ± 0,17	60.90 ± 9 97	342,45	4,54	11,77	288,3
MSG 2 %	3,18 ± 0,53	1,33 ± 0,17	66,11 ± 11,36	325,40	4,76	15,10	309,8

** $P < 0.05$

Table 30. Nervous Central System Cellularity (2nd Generation) (8353)

Vitamins 2 %

	RNA mg/g tissue	DNA mg/g tissue	Protein mg/g tissue	Nucleus number (millions)	Cellular size (nucleus weight) mμg	RNA / nucleus mμg	Protein / cell mμg
AT BIRTH							
Control	4,29 ± 0.16	2,63 ± 0,38	36,26 ± 7,80	98,97	2,30	10,26	86,3
MSG 1 %	4,14 ± 0,45**	2,80 ± 0,24	36,51 ± 3,71**	104,08	2,23	9,17*	81,1
MSG 2 %	4,17 ± 0,32***	2,85 ± 0,32	35,31 ± 5,67**	107,52	2,20	9,15***	77,3
AT 15th DAY							
Control	....	....	....	....	....	....	....
MSG 1 %	3,17 ± 0,50	1,32 ± 0,15	37,41 ± 3,19**	229,80	4,75	15,03	177,6
MSG 2 %	3,80 ± 1,70	1,42 ± 0,60	37,24 ± 18,02*	201,22*	4,85	16,48	161,2
AT WEANING							
Control	....	....	....	....	....	....	....
MSG 1 %	2,71 ± 0,50	1,35 ± 0,28	38,71 ± 4,67***	293,71	5,07	13,46	194,2***
MSG 2 %	2,13 ± 1,14	1,06 ± 0,43	29,55 ± 8,34***	231,21	6,92	11,92	186,2**

*** P < 0,001; ** P < 0,01; * P < 0,05; • significance limit.

Nevertheless the authors concluded that the "reduced hyperplasia" (sic) of the CNS in the MSG groups "cannot be considered a negative element," and that they disappeared in the second generation. They considered that "contrary to the effects following parenteral administration, no evidence exists supporting toxicity after administration of MSG in the diet" (8353).

11. Pradhan and Lynch in 1972 (5884) provoked a delayed behavioral deficit in rats with MSG. Holtzman littermate neonates were intubated with 10 ml water or MSG 1.25, 2.5, or 5.0 g/kg daily on days 5-10 of age.

After three months, deficiencies of weight gain were related to dose but not to intakes. Table 31 summarizes the behavioral data for 22 rats, and the authors pointed out that:

(1) Only the 5 g/kg dose significantly decreased spontaneous motor activity.

(2) Only rats given the 5 g/kg dose had significantly few correct scores at the 10th session of maze-learning. All MSG rats took significantly longer than controls ($\underline{P}<0.01$) to run the maze, and the 5 g/kg group took longest.

(3) Only the controls and the 5 g/kg rats were trained for fixed-ratio food reinforcement. Neither their responses nor the effects of amphetamine on their responses differed significantly.

The authors cautioned against extrapolation of the maze-learning effects of 5 g/kg of MSG to human infants.

12. In 1973 Johnston (3436) compared the convulsant activity of L-glutamate with those of: N-methyl-D- and L-aspartate, ibotenate, β-N-oxyalyl-L-α, β-diaminoproprionate (ODAP), DL-homocysteate, D-and L-glutamate, L-aspartate, α-aminobutyric acid, glycine, DL-C-allylglycine, DL-methionine-DL-sulfoxime, and N-oxalyl-β-alanine.

Table 31. Behavioral Activities in Normal and MSG-Pretreated Rats (5884)

Behavioral situations	Dose (g/kg) group[a]			
	0(6)	1.25(4)	2.5(6)	5(6)
Spontaneous activity[b]	933±48	901±100	832±69	750±50[e]
Maze running[c]				
score	92±2	85±6	83±5	47±12[f]
Latency (sec)	4.6±0.2	9.1±1.5[f]	6.0±0.4[f]	9.3±1.7[f]
FR (food) reinforcement[d]				
Number of responses	1405±219			1085±230
Effect of amphetamine	67±17			54±15

[a] Figures in parentheses indicate number of rats in each group, except for FR reinforcement in which 5 rats were in each group.

[b] Average of 5-day data during the second week of trial.

[c] Data from the 10th session.

[d] Data on responses are from the 19th session. Effect of amphetamine (0.5 mg/kg) on responses from a subsequent session is given as percent change from control.

[e] $P<.05$ compared to controls.

[f] $P<.01$ compared to controls.

Ten-day-old rats (16-23 g BW, number and sex not stated) and adult rats (180-200 g BW) were given i.p. injections of these compounds in neutral aqueous solutions (up to 1 M) in doses ranging from 0.5 mmoles/kg to 20 mmoles/kg. Controls were given NaCl solution. All of the amino acids tested produced convulsions with repeated tonic seizures, and onset was dose-dependent. L-aspartate and D- and L-glutamate were inactive at doses of 10 mmoles/kg but convulsant 30 minutes after 20 mmoles/kg (see Table in original paper for details). The most powerful convulsant was N-methyl-D-aspartate when given to 10-day-old rats, but none of the excitant amino acids produced convulsions in the adult rats, and neither did GABA nor glycine.

These data were interpreted by the author as supporting Olney's (cited) thesis that the neurotoxic and excitant properties of the acidic amino acids were directly interrelated. The author concluded that "any excitant amino acid should be suspect as a food additive."

C. Gerbils.

Bazzano et al. (0581) reported in 1970 that when large amounts of glutamate (30 g/kg per day) were fed to 56 mature male Mongolian gerbils, they observed no symptomatic increase or decrease of visual acuity or other neurological activity (only behavioral criteria are mentioned), except that these gerbils were more tranquil than controls. They concluded that the blood brain barrier protected adults against neurological effects of very high oral doses of MSG. (see also pp. 114 and 174).

D. Chicks

1. In 1967 Kramer et al. (4025) investigated some effects of GABA or MSG, given i.p. on the EEG and ERG of fifteen 2-8-day-old sex-linked hybrid cockerels. In unrestrained chicks MSG produced:

(1) An EEG typical of natural sleep, accompanied by wing-droop and other postural changes unlike those seen during roosting, and by "behavioral depression."

(2) Both components of the tectal evoked response to light were depressed by GABA 1.75 g/kg, and were blocked by GABA 3-4 g/kg or by MSG 4 g/kg. With MSG this was seen before, but not during, related ERG changes in which the a-wave was depressed or blocked, the b-wave was augmented, and the c-wave was unaltered. The MSG had little effect on the "off" response.

The authors declined to guess at where the blockade occurred.

2. In 1971 Shimizu et al. (6714) tested a theory that the Na component of MSG would be nephrotoxic to chicks. Drinking water containing MSG 2.6%, NaCl 0.9%, or MKG 2.8% (standardized for Na or K ion) was offered to 2-day-old hybrids for four weeks. One group was offered MSG 1.3%. Results were:

(1) Twenty-two of 30 offered MSG 2.6% died in three days, the rest in five. Autopsies and analyses (given in detail) revealed widespread gouty deposits and renal glomerular degeneration. With 1.3% MSG 2 of 30 died, and renal atrophy was found in 10 of 28 when killed on day 35.

(2) Only 8 of 20 offered NaCl died, and renal tubular degeneration was diffuse, without glomerular lesions.

(3) No deaths or pathology occurred in 19 chicks offered MKG, or in 31 offered plain water.

The authors concluded that the glutamate as well as the Na of MSG had contributed to extremely rapid formation of uric acid, and they urged further study of this phenomenon even though avian gout was not directly related to human gout. [But see p. 123]

3. Snapir et al. (6643) in 1971 produced brain lesions in the hypothalamic region of chicks with MSG. Eighty-four 5-day-old New Hampshire X White Leghorn cross-bred males were injected s.c. in the dorsal part of the neck as in Table 32. Three birds from each group were decapitated 40 days after the start of the injections, and brains were fixed without perfusion.

Brain lesions were found in all MSG and in no control chicks. "The damage was so obvious, that it could even be detected with the naked eye." It involved the hypothalamus but not the ventromedial nuclei, and consisted of neuronal necrosis and reduced populations. Phagocytosis was less complete than in Olney's mice (5488 and 5490 cited). Appetite and growth were unaffected (6643).

4. Carew and Foss (1155) in 1971 studied the toxicity of single s.c. injections of MSG in day-old broiler chicks fed a commercial ration.

(1) Treatments and findings are shown in Table 33, from which the acute data were reported on pp. 47-49; the authors concluded that the Na did not contribute to the acute toxicity of MSG.

(2) In a second experiment groups were injected s.c. daily for ten days with MSG 0.125, 0.25, 0.5 and 1.0 g/kg (plus control groups). No significant differences were found in food intakes or growth at eight or 16 weeks. The authors commented that chicks and mice responded differently to MSG s.c.

(3) In a third study diets containing 0.5-10.0% MSG were fed from one to ten days of age; and in a fourth study 0.5-2.5% MSG was fed from day 1-28. Mortality, intakes, and growth were unaffected during 16 weeks.

E. Monkeys

1. In 1969 Olney and Sharpe (5490) reported the first observation of a brain lesion produced by MSG in an infant monkey (Macaca mulatta). The male,

Table 32 (6643)

	Treatment		Volume (ml)
Group 1	1 mg MSG[a]/g BW	Single inj.	0.3
Group 2	1 mg MSG[a]/g BW	One daily inj./10 days	0.3
Group 3[b]	4 mg MSG/g BW	Single inj.	1.2
Group 4[c]	4 mg MSG/g BW	One daily inj./8 days	1.2
Group 5	Saline	Single inj.	0.3
Group 6	Saline	One daily inj./10 days	1.2

[a] MSG manufactured by B.D.H. Chemical Division, England.

[b] Three chicks died 24 hr postinjection.

[c] Five chicks died during the treatment.

Table 33. Tolerance of Chicks for a Single Subcutaneous Dose of Monosodium Glutamate (MSG) (1155) (Experiment 1)

	Injection treatment (per g body weight)	Mortality[a, b] (0-7 days) No. Chicks
1.	Control (no injection)	0
2.	0.9% NaCl[c]	0
3.	1 mg MSG	1 (6.3)
4.	2 mg MSG	1 (6.3)
5.	3 mg MSG	5 (31.3)
6.	4 mg MSG	13 (81.3)
7.	5 mg MSG	16 (100)
8.	NaCl equimolar to trt. 3	0
9.	NaCl equimolar to trt. 4	2 (12.5)
11.	NaCl equimolar to trt. 5	0
11.	NaCl equimolar to trt. 6	0
12.	NaCl equimolar to trt. 7	3 (18.7)

[a] Sixteen day-old chicks started per treatment.

[b] Figure in parentheses shows percent mortality.

[c] Injections equal in volume to treatment 7.

eight hours old, appeared alert and healthy but was judged premature from its small size (260 g, 16.5 cm crown to rump).

The monkey was killed 3 hours after s.c. injection of MSG 2.7 g/kg (in water, vol. 2.8 ml), having shown no signs of CNS disturbance.

Nevertheless, a lesion similar to those of mice, affecting the periventricular-arcuate region of the hypothalamus, was seen by light microscopy. Electron-microscopy revealed involvement mainly of dendrites and cell bodies of neurons; glial and vascular components were unaffected.

The authors commented that:

(1) The lack of signs in this monkey, while a small percentage of its brain cells were being destroyed, indicated that such a process might go unrecognized in a human infant under routine circumstances.

(2) However, the damaging dose had been high, and had been given s.c. rather than orally. Whether damaging circumstances could be triggered naturally remained to be seen; and there were many factors that could raise blood glutamate levels, presumably a "prerequisite to lesion formation." (5490).

2. A controversy ensued (4481,8254,5491). Criticisms were:

(1) Heavy ionic concentration could be damaging per se (4481).

(2) No chemical controls such as inorganic Na salts or Na salts of other amino acids (4481).

(3) No passage through the gut mucosa (8254).

(4) No data on blood glutamate level (4481).

(5) Only a single case (4481,8254).

(6) The monkey was premature (8254).

(7) No human infant could ingest so much MSG from infant diets (4481).

(8) "Mothers with infants in arms" would be scared incalculably (8254).

In reply to points (7) and (8), Olney and Sharpe (5491) stated (7) that they had not given the monkey the lowest effective dose, for which there were no data except in mice (see below); and (8) had there been any evidence on safety levels, there would be no controversy.

In reply to points (1-4) Olney and Sharpe (5491) reported new findings cited as "unpublished" or "in preparation:"

(1) NaCl 8 g/kg (25 times the Na content of MSG 1 g/kg) had prostrated infant mice for four hours but produced no hypothalamic or retinal neuropathology (HRN). On the other hand purified glutamic acid 1 g/kg by intubation had produced hypothalamic neuronal necrosis similar to that in the monkey.

(2) HRN had not been produced in mice by the following at 3 g/kg: L-serine, L-glycine, L-alanine, DL-methionine, L-phenylalanine, L-proline, L-leucine, L-arginine, L-lysine. HRN had been produced by L-cysteine, sodium L-aspartate, sodium DL-α-aminoadipate, all both orally and s.c. Monosodium glutarate 5 g/kg had produced no HRN (5481 cited).

(3) MSG 10% in water had been fed by tube to 10-day-old mice. Hypothalamic damage had occurred in 54% (n = 24) at 0.5 g/kg and 100% (n = 19) at 1.0 g/kg. When MSG 0.5 g/kg and aspartate 0.5 g/kg were given together, the damage was "consistently more severe" than with either compound alone.

(4) MSG 0.5-1.0 g/kg tube-fed to 23 infant mice had produced blood glutamate levels often over 50 mg/100 ml by 15-30 minutes, which returned to normal (5 mg/100 ml) within two hours.

3. In 1971 Abraham et al. (0032, see also p. 76) gave four 4-day-old rhesus monkeys (M. mulatta) MSG 4 g/kg, two s.c. in the interscapular region,

and two by intubation. Two controls were given H_2O s.c. and by tube respectively. They were killed after 3 or 24 hours, and brains were fixed without perfusion for light microscopy; samples were post-fixed for electronmicroscopy.

Light microscopy failed to show any adverse effect of MSG on the lateral preoptic nuclei, arcuate nuclei, or median eminence. Electronmicroscopy showed normal hypothalami not differing in any way from the controls (0032). Since the authors had found differences in mice (see p. 76) they inferred a species difference possibly based on the rate of development of CNS myelinization.

4. In 1971 Reynolds et al. (6095) fed MSG to 16 infant monkeys (M. mulatta and M. irus) by nasogastric tube, and H_2O to five controls. Doses were 1, 2 and 4 g/kg, as 50% solutions. The monkeys were killed after six hours by perfusion-fixation (described in full).

No significant differences in the periventricular-arcuate region were found by light or electronmicroscopy.

The authors commented that areas resembling Olney's description of lesions were found but were identified as poorly fixed tissue, and that they, too, had confirmed genuine lesions in MSG-treated mice (cited as "in preparation"). Newborn mice and monkeys were "hardly comparable", and differences in glutamate metabolism or susceptibility were conceivable (6095).

5. In 1972 Olney et al. (5485) reported on 9 M. mulatta infants (Table 34). Six were full-term and healthy infants, H and I had mother problems, and A was reported previously (5490, on pp. 99, 102). This is a 25-page report (5485) with details of methods, findings, and a review of the issues.

The experiment is shown in Table 34. Oral doses were fed by nasogastric tube in 20 ml/kg of 50:50 water: skim-milk; and s.c. doses were injected in multiple sites as 25% MSG or 10% NaCl. All infants except A were killed by

Table 34 (5485)

	Age (days)	Sex	Wt (g)	Dose (g/kg)	Dose mmoles/kg	Compound	Route	Acute symptoms	Blood glutamate (mg%) (at 1/2 hour intervals) 0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
A	0.5	M	260	2.7	16	MSG	s.c.	---	Not studied										
B	7	F	507	2.7	16	MSG	s.c.	Vomiting	12.5	70	70	70	70	60	60	60	55	50	30
C	7	M	430	1.0	6	MSG	Oral	---	7.5	12	12	20	12	12	12	12	10	7.5	7.5
D	7	F	330	2.0	12	MSG	Oral	---	7.5	10	7.5	7.5	20	12.5	7.5	7.5	7.5	7.5	7.5
E	7	M	507	4.0	24	MSG	Oral	Vomiting	3	5	30	30	30	12.5	12.5	20	12.5	12.5	10
F	8	M	473	0.3	6	NaCl	Oral	---	7.5	10	7.5	7.5	7.5	3.0	5	5	5	5	5
G	7	M	440	0.6	12	NaCl	Oral	---	12.5	7.5	7.5	7.5	5	5	5	5	5	5	5
H	4	M	406	1.2	24	NaCl	s.c.	Cyanosis	12	12	10	12	12	20	25	25	20	20	12
I	4	F	352	4.0	24	MSG	s.c.	Cyanosis vomiting convulsions	20	50	80	100	100	100	100	100	90	80	80

perfusion-fixation five hours after treatment. Blood glutamate analyses were performed blind.

The major findings were:

(1) All the MSG-treated infant monkeys developed hypothalamic lesions that were similar in cytopathological detail to those described in infant rodents receiving similar MSG doses.

(2) The hypothalami of the three NaCl-treated controls showed no such cellular pathology.

(3) Relatively low oral doses (1 and 2 g/kg BW) produced small focal lesions located primarily in the rostro-ventral aspect of the infundibular nucleus of the hypothalamus.

(4) The high s.c. doses given infants A and B (2.7 g/kg) and I (4 g/kg) (see Table 34) caused lesions which spread throughout and sometimes beyond the infundibular nucleus.

(5) Degeneration was more advanced at five hours than at three hours. Some cell bodies appeared dark and condensed, others swollen and with pyknotic nuclei.

The infundibular nucleus was defined as the primate equivalent of the rodent arcuate nucleus. The authors stated that poor fixation could not explain the juxtaposition of necrotic neurons and well-preserved other tissue components (citing 5485, Fig. 3b).

The blood glutamate values (Table 34) were judged to correspond with MSG dosages and also with observed pathology. The high basal value for infant I was "probably" explained by its initial dehydration, but the response in H was "inconsistent with expectation."

The acute reactions of vomiting and convulsions (Table 34) were interpreted as both dose and age related, though uncommon in infant primates. Examples

were cited in mice, dogs cats, humans, and primates including vomiting from MSG 1 g/kg s.c. in adult rhesus (cited as Olney, unpublished; see also pp. 83-85 and 194)..

The authors discussed the discrepancies between their findings and those outlined on pp. 103-104 (0032, 6095).

They commented that Reynolds et al. (6095) had prepared tissue for light microscopy by a method earlier reported as unsatisfactory (1053 cited) and that their spot samples for electronmicroscopy could have missed any focal lesions [this is reported to have been admitted (2502)]. Also, "in discussing their data at a recent meeting (25), W.A. Reynolds mentioned that some of their infants vomited an unknown portion of the administered dose" (5485, p.481).

The authors criticized the report by Abraham et al. (0032) for illustrating only a 24-hour photomicrograph, on which negative findings would be expected, and no earlier micrographs. This, their own finding that an oral dose of MSG 4 g/kg was emetic for primates, and the high yield of negative findings in mice given MSG 4 g/kg s.c. by Abraham et al. (0032, see p. 76), all suggested "some repeating defect(s)" of timing or technique (5485).

The authors drew the following conclusions from their evidence:

(1) Primates were susceptible to MSG neurotoxicity, but it was still questionable whether low oral doses were the direct causes of related small lesions.

(2) The average blood glutamate threshold for occurrence of lesions in infant primates was estimated to be 20 mg/100 ml.

(3) The liability of MSG-lesioned infant primates to endocrine disturbances in adulthood remained uncertain for lack of evidence. The authors believed that infants A, B, and I (Table 34) would have been liable,

and they cited additional reasons for that belief (see p. 485 of original paper).

(4) Brain damage resulted from lower doses than were needed to produce overt behavior symptoms. This removed the basis for the "assumption" by the NAS Subcommittee (5226 cited) that human infants were not vulnerable to MSG neurotoxicity.

(5) To MSG exposures should be added free glutamate, aspartate, and cysteine, present in protein hydrolysates and with additive neurotoxicities. These were found in adult foods such as bouillon cubes often given to infants. The possible risks of dysfunction from over-accumulation of these natural metabolites should be considered, not dismissed. [See pp. 214 onward.]

IV. Toxic Effects: Human

Several phenomena have been reported in certain humans as associated with consumption of MSG. Allergic symptoms have been described in individuals sensitive to beets after ingesting MSG prepared from the waste liquor of sugar beet processing mills. Also, the so-called Chinese Restaurant Syndrome (CRS) was first recognized as a diagnostic entity in 1968 by Kwok (4197) and by Schaumburg and Byck (6470). The CRS symptoms are reported to be transient, to range from numbness and general weakness to sensations as in an acute myocardial attack, and to appear most markedly in susceptible individuals who eat food containing MSG on an empty stomach.

1. One of the sources of MSG is Steffen's liquor, a beet residue following the extraction of the sugar (7476). In 1950 Randolph and Rollins (5997) reported that individuals sensitive to beets were also sensitive to MSG derived from beets. The list of possible signs included rhinitis, urticaria,

atopic dermatitis, asthma, gastrointestinal upset, and also fatigue, allergic myalgia, and other allergy-related symptoms.

The authors added that people who were highly and specifically sensitive to corn and wheat were also sensitive to MSG derived from corn and wheat gluten. They claimed that because of inexact Federal labeling regulations pertaining to commercially processed foods, the specific avoidance of allergenic ingredients was difficult for persons with particular allergies (5997).

2. The first reports of physical distress following the consumption of food prepared in Chinese restaurants appeared in a series of letters in the New England Journal of Medicine in 1968 (4197,6469,3537,0700,4872,2599,6470, 0196). The letter containing the first hint that it might be the MSG used in Chinese restaurant cookery which caused the symptoms observed by the writer in himself and in several of his Chinese friends, both medical and nonmedical, was written by a pediatrician, Dr. Robert Ho Man Kwok (4197). He felt numbness at the back of his neck gradually radiating to both arms and his back, palpitations, and general weakness. The syndrome began within 15 to 20 minutes after eating the first dish and continued for about two hours with no after-effects.

Kwok's letter in the April issue was followed by correspondence in the May and July issues expanding the spectrum of symptoms to include:

(1) Tightening of the masseter and temporalis muscles, lacrimation, periorbital fasciculation, palpitations and syncope (6469).

(2) Numbness of the jaw (3537).

(3) Temporal headache (0700).

(4) Vicelike pounding and throbbing in the head (4872).

3. Gordon (2599) described the experiences of seven people known to have reactions to Chinese food. Seventeen minutes after beginning to eat Chinese food the following were noted:

(1) Sudden tightening of the face, along with unilateral numbness, usually strongest near the zygoma.

(2) Some weakness of the mouth.

(3) Dizziness accompanied by flushing and sweating.

(4) In some persons, a "bandlike headache with orbital pain."

(5) In others, facial anesthesia crossing the dermatome midline pattern.

(6) Sinus tachycardia reaching 100 beats per minute.

(7) One physician suffered such intense heavy aching in his biceps and triceps 45 minutes after eating a Chinese dish that he was convinced he was having an acute myocardial infarction.

(8) Another physician experienced extreme fatigue of the shoulder girdle "simulating a palsy."

(9) A third had paresthesia and weakness in the upper extremities intense enough to suggest paralysis.

In the July issue of the journal Schaumburg and Byck (6470) identified MSG as the cause.

4. Ambos et al. (0196) found that susceptibility appeared to be more frequent among women than among men. Furthermore, susceptible females were more sensitive than susceptible males, requiring half the dose of MSG (2 tsp MSG per 6-oz glass of tomato juice) to provoke symptoms.

5. In 1969 Schaumburg et al. (6471) reported a study of the acute human pharmacology of MSG.

First, they showed that MSG was the causative factor in CRS by demonstrating that 200 ml of wonton soup containing 3 g of MSG provoked an attack in two subjects previously identified as sensitive. No attack was provoked by wonton soup prepared in the same restaurant without MSG or by any of its components tested individually. In addition, a blind procedure with four sensitive subjects

eating this soup in the same restaurant provoked attacks in amounts of 3 g MSG or less.

Second, they were able to induce attacks in the original two subjects with monopotassium L-glutamate (4 g), DL-glutamic acid (5 g), and L-glutamic acid (5 g). Monosodium D-glutamate (7 g), monosodium L-aspartate (5 g), sodium chloride (10 g), and glycine (5 g) caused no reactions.

The oral threshold dose of MSG for minimum symptoms in 36 subjects was 1.5-12 g and the time lag was 15-25 minutes (Fig. 2). Threshold was unrelated to sex, BW or age (of adults). Intensity and duration were dose-related in six subjects (Fig. 3). However, symptoms were hard to separate and to sequence because changes were gradual.

When 13 subjects were given MSG i.v. three major categories of symptoms emerged: (1) burning, (2) facial pressure, and (3) chest pain. Some subjects experienced headache. The i.v. threshold was 25-125 mg and the time lag 17-20 seconds. Category (1) came first. Category (3) came 5 seconds later and showed no EKG changes. Category (2) usually appeared last, with durations of 30 minutes after oral doses and 120-180 seconds after the (smaller) i.v. doses.

When MSG was given to two subjects i.v. after inflating an axillary cuff, the "burning" sensation was shown to be peripheral; it took 17 seconds after deflation to spread to chest and neck.

CRS symptoms were produced in 55 of 56 "normal" subjects (30 males, 25 females) 21 to 67 years old who were given MSG orally; the 56th had no reaction to MSG 21 g orally but reacted to 50 mg i.v. The authors could produce symptoms at some dose level in all subjects tested, and all who had complained of the syndrome were found to have oral thresholds of 3 g or less.

In three families with more than one susceptible member no known genetic pattern could be established.

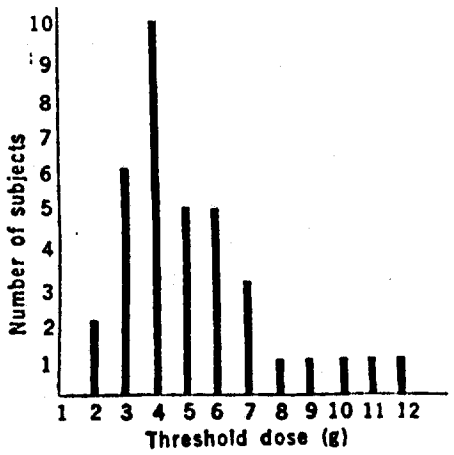


Fig. 2. Oral thresholds for minimum symptoms of MSG response in 36 subjects. (6471)

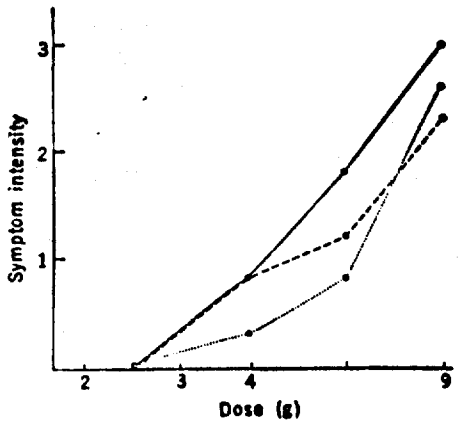


Fig. 3. Relation between intensity (see text) of MSG response and oral dose in grams. Solid line, burning; dashes, facial pressure; and dotted line, chest pressure. Each point represents a mean intensity from four or more responses. The data were obtained from six subjects. (6471)

The authors concluded that their studies ruled out any contaminant in commercially manufactured MSG as an important cause of symptoms. They were unable to arrive at a mechanism of action, in a lengthy discussion of possibilities. They stated that although MSG had been classed as "GRAS" it could "produce undesirable effects in the amounts used in the preparation of widely consumed foods."

6. In a follow-up letter to the New England Journal of Medicine (1091) Byck and Schaumberg recommended that since MSG was not a nutritional necessity but was used too commonly for specific human responses to be readily recognized, "it would be appropriate to remove MSG from the GRAS list and establish safe levels of MSG intake during pregnancy and infancy."

7. In 1970 Morselli and Garattini (5084) reported that 24 volunteers (17 males, 7 females) aged 18-34 were given 150 ml of beef broth with or without 3 g MSG, followed by other dishes, using double-blind and crossover techniques. Subjects then scored their sensations every 20 minutes for three hours on a form listing eleven symptoms and three levels of intensity. At the same time pulse rates, blood pressures, and respiration rates were recorded.

The authors reported that no significant differences were found, in their objective measurements or the subjective scores, between MSG and control groups, that nobody reported a "burning" sensation, and therefore, that MSG "does not provoke any symptoms" of CRS.

Furthermore, they criticized Schaumberg et al. (6471 cited) for using "a very small number of subjects" (sic), high doses, a "questionable" (sic) route of administration, and inadequate statistical methods.

Himms-Hagen (2993) objected that the above study (5084) used only non-susceptible subjects, and a sex ratio that was opposite to the reported susceptibility ratio. Such a study would "obviously" not reveal significant reactions.

He added that he personally was susceptible and was capable of recognizing when he had ingested MSG and also how much.

8. In 1970 Bazzano et al. (0581, see also p. 97) fed 11 adult male subjects 24-147 g of MSG per day for 14 to 42 days, along with a chemically defined diet that supplied the essential amino acids in optimum amounts so that glutamic acid was the sole source of nonessential nitrogen, and calories to maintain BW. A lesser amount of this diet was fed to three other subjects along with MSG. Controls were fed an amino acid formula diet only. The original aim was to study a hypocholesteremic effect of glutamic acid.

The reduction of plasma cholesterol (Table 35) averaged 42 ± 5.8 mg/100 ml, and there was a parallel fall in β-lipoproteins. No changes were observed in hepatic or neurologic function, weight, irritability, appetite or mentation. All subjects tolerated the diet; none developed CRS.

The authors commented that to their knowledge, no permanent neurotoxic effect for glutamate had been definitely reported for adult humans. They referred this to the blood brain barrier, but conceded that the problem of the age of transition from susceptibility to tolerance for MSG in man remained open. They commented that the lipid-lowering effect of MSG was "clearly" metabolic. [See also p. 174.]

9. In 1971 Ghadimi et al. (2474) hypothesized that the CRS syndrome was mediated by acetylcholine. To establish a baseline they took 14 fasted volunteers (10 males, 4 females) and fed them MSG 150 mg/kg in 150 ml water. All developed CRS within 5-35 minutes. As a control, three subjects were given histidine loading tests (150 mg/kg).

They then studied repression of CRS by diminishing subsequent MSG treatments or by giving atropine s.c. or i.m. They augmented the CRS by increasing MSG doses or by giving prostigmine. They routinely determined blood glutamate

Table 35. Effects of Glutamate Feeding in Human Subjects (0581)

Subject	Time (days)	Dose (g/day)	delta-Cholesterol (mg/100 ml)[a]
J.B.	34	147	-27
R.S.	28	88	-82
R.M.	27	147	-16
J.M.	21	137	-46
P.B.	21	137	-40
P.K.	21	147	-55
J.A.[b]	14	25	-16
	14	50	- 7
	21	100	+ 3
S.S.[c]	22	131	- 5
R.M.	21	133	-23
M.E.[c]	21	126	-20
M.E.	21	68	-41
	21	137	-42
G.R.	28	137	-29
W.B.	42	137	-75
D.M.	21	147	40

[a]delta-Cholesterol represents the difference in concentrations of plasma cholesterol from those in subjects on the control diet. None of the subjects showed any clinical effects.

[b]Monosodium glutamate was added to a house diet.

[c]Monosodium glutamate was added to a low protein (25 g) diet.

levels but did not report them (explicitly); they also determined blood cholinesterase, amino acids and, in some samples, CO_2, pH, and bicarbonate (found to be normal). See the paper itself (2474) for details. The range of MSG doses was 25-250 mg/kg.

The subjective manifestations of CRS were like those already described plus some individual reactions that included thirst and gastrointestinal distress. Five subjects given EKGs showed normal tracings. Similarly, blood pressures and pulse rates were found to be normal.

Reduction of the MSG dose reduced the CRS reactions, and so did atropine even when the MSG dose was increased. Increase of the MSG dose to 250 mg/kg, or prostigmine without increased MSG, intensified the CRS reactions. Histidine 150 mg/kg did not affect plasma cholinesterase activity, but MSG 150 mg/kg reduced it between 20 and 90 minutes after the dose, with peak reduction of 30% at 60 minutes.

The authors commented that glutamate was known to be convertible to acetylcholine in the presence of extracellular Na^+ and that the CRS symptomatology was consistent with neuroexcitation from transient rapid elaboration of acetylcholine and with the observed patterns of cholinesterase activity (degradation of acetylcholine). They suggested that CRS should be called "transient acetylcholinosis."

10. In a letter to the New England Journal of Medicine, Upton and Barrows (7596) reported that an epileptic patient on diphenylhydantoin therapy developed CRS after eating food containing MSG. They advised patients on diphenylhydantoin therapy to avoid foods rich in MSG, particularly wonton soup.

11. In 1972 Kenney and Tidball (3676) studied human susceptibility to MSG. In a pilot experiment they confirmed the absence of objective signs (pulse, blood pressure, EKG, respiration, and also skin temperature during

"burning"). In a second pilot experiment (uncontrolled) they found reactions extremely variable in individuals, and uncorrelated with MSG doses; subjects had been conditioned by the statement of informed consent, and the authors questioned the validity of subjective susceptibility studies. They then undertook a placebo-controlled study in two phases.

In phase one 77 normal volunteers (44 males, 33 females) were given 150 ml tomato juice which contained 5 g MSG on one day and 0.8 g NaCl to adjust the taste on the other two days. The purpose of this first experiment was to detect positive reactors at the 5 g MSG dosage level. Twenty-five persons reported symtoms after taking MSG. There was a significant ($P < 0.01$) difference between the frequency of occurrence in females and males (42% females vs. 25% males). Blood glutamate levels rose after MSG, but were not influenced by race, by the presence or absence of reactions, or by the susceptibility of the subjects.

In phase two, 22 of the 25 subjects reporting reactions were given 150-ml solutions containing at random 1, 2, 3, 4, or 5 g of MSG or 0.8 g NaCl at random on each of three days in a double-blind experimental pattern. Eight series of experiments were performed.

The results (see 3676 for details) suggested three classes of symptoms:

(1) Not dose-dependent - heartburn, gastric discomfort, weakness in limbs, and possible lightheadedness.

(2) Highly dose-dependent - stiffness-tightness symptoms that might appear at low levels.

(3) Dose-dependent above an appearance threshold - pressure symptoms, warmth, burning, perhaps also tingling and headache.

The authors commented that the reactions were peripheral, which suggested involvement of "subcutaneous free nerve endings of primitive chemical sense," and that MSG might not be the direct effector. It seemed likely that the

glutamate in MSG was not physiologically equivalent to that ingested in protein. Thus although a typical protein-glutamate intake might be 40 g/day and MSG intake 1 g/serving, the latter produced symptoms in some subjects; in a few, at less than 2 g/serving, but in perhaps 30% at 5 g (3676).

12. Rosenblum et al. (6242) in 1971 used single and double blind studies to observe the effects of oral administration of varying doses of MSG to 99 male subjects (Table 36 describes the experimental design.). The results are shown in Table 37 for 5 g MSG and in Table 38 for 8 g and 12 g MSG.

The two commonest complaints when 5 g MSG were administered were light-headedness or dizziness, and tightness of the face (18%). With doses of 8 g or 12 g MSG, nausea was more frequent than at the lower dose.

The authors stated that none of their subjects reported the triad known as CRS. They found that complaints were questionnaire-dependent.

13. Levey et al. (4364) studied the i.v. administration of:

(1) Five different amino acid preparations of known glutamic acid content (three protein hydrolysates, two amino acid mixtures) to 47 male subjects.

(2) Solutions containing partially neutralized glutamic acid to 31 male subjects.

(3) An amino acid mixture with a quantity of glutamic acid added sufficient to make the total glutamic acid content equal that in the casein acid hydrolysate also used in the experiment, to 11 male subjects.

They found that:

(1) The nausea and vomiting produced paralleled the free glutamic acid content of the administered preparations.

Table 36 (6242)

Experimental Design

Group I, 24 subjects received 5 g MSG in tap water	Group II, 25 subjects received 5 g MSG in tap water	Group III, 25 subjects received 5 g MSG in chicken stock	Group IV, 24 subjects received 5 g MSG in chicken stock

Group V, 24 subjects received 1.7 g NaCl in chicken stock	Group VI, 25 subjects received chicken stock

Group VII, 11 subjects, 6 received 8 g MSG in chicken stock, 5 received 2.8 g NaCl in chicken stock

Group VIII, 10 subjects, 5 received 12 g MSG in chicken stock, 5 received 4.2 g NaCl in chicken stock

Table 37 (6242)

Complaints Reported After Ingestion of 5 g MSG or Control Substances[a]

Complaint	GP I (25) MSG 5 g		GP II (25) MSG 5 g		GP III (24) MSG 5 g		GP IV (24) MSG 5 g		Total (98) MSG		Gp V (24) NaCl 1.7 g		GP VI (25) stock		Total (49) control	
	Yes	%	Yes	%	Yes	%	Yes	%	Yes	%	Yes	%	Yes	%	Yes	%
Headache	3	12	5	20	3	13	6	25	17	17	3	13	1	4	4	8
Nausea	-	-	1	4	-	-	6	25	7	17	-	-	-	-	-	-
Nervousness	1	4	1	4	1	4	-	-	3	3	-	-	2	8	2	4
Itchy	2	8	1	4	1	4	1	4	5	5	1	4	-	-	1	2
Lightheadedness	4	16	10	40	2	8	9	38	25	26	2	8	2	8	4	8
Flushed	-	-	4	16	-	-	5	21	9	9	1	4	1	4	2	4
Heart palpitations	1	4	2	8	-	-	-	-	3	3	-	-	-	-	-	-
Heartburn	-	-	2	8	-	-	1	4	3	3	-	-	-	-	-	-
Grogginess	1	4	4	16	1	4	5	21	11	11	1	4	-	-	1	2
Cold and clammy	-	-	-	-	3	13	-	-	3	3	-	-	-	-	-	-
Sweating	-	-	7	28	-	-	2	8	9	9	-	-	-	-	-	-
Dry mouth	2	8	6	24	2	8	1	4	11	11	2	8	1	4	3	6
Tightness in face	2	8	5	20	3	13	8	33	18	18	2	8	1	4	3	6
Sour stomach	-	-	2	8	-	-	1	4	3	3	-	-	-	-	-	-
No symptoms	15	60	5	20	16	67	7	29	43	44	17	71	20	80	37	76

[a]Groups I and II, the MSG was dissolved in 100 ml of tap water, groups III and IV in 100 ml of soup stock diluted 1:1 with water. Group V received NaCl in 100 ml of soup stock and group VI received 100 ml of diluted soup stock. Dashes indicate that no complaints were reported.

Table 38 (6242)

Symptoms[a] Reported After Ingestion of 8 or 12 g of MSG

Symptoms[a]	MSG 8 g GP VII(6) Yes	NaCl 2.8 g GP VII(5) Yes	MSG 12 g GP VIII(5) Yes	NaCl 4.2 g GP VIII(5) Yes
1. Burning sensation radiating out to forearms and thorax	-	-	-	-
2. Infraorbital tightness	1	-	3	1
3. Substernal pain	2	-	-	-
4. Headache	3	2	2	-
5. Fullness in stomach	1	-	2	2
6. Vomiting	-	-	-	-
7. Nausea	1	-	2	-
8. Diarrhea	-	-	-	-
9. Flushing	1	-	-	-
10. Dizziness	1	1	1	1
11. No symptoms	1	4	2	3

[a]Symptoms 1-4 reported by Schaumburg et al [Science 163: 826-828 (1969)] and Schaumburg and Byck [N. Engl. J. Med. 279: 105 (1968)], symptoms 5,6,8,9, and 10 reported by Albert et al [J. Nerv. Ment. Dis. 104: 263-274 (1946)], and symptom 7 reported by Himwich et al [J. Nerv. Ment. Dis. 121: 40-49 (1955)]. Dashes indicate that no complaints were reported.

(2) There was a direct relationship between the free glutamic acid of the serum and the occurrence of toxic effects following administration of the amino acid preparations.

(3) Nausea and vomiting were produced in more than half of the subjects when the serum free glutamic acid level reached 12-15 mg per 100 ml (see p. 194).

(4) More nausea and vomiting were seen when glutamic acid was given i.v. incorporated in an amino acid mixture than when partly neutralized glutamic acid was given i.v. alone. The authors concluded that the toxicity of glutamate might be potentiated by other amino acids.

14. In a 1972 symposium paper, Olney (5482) discussed two contrasts:

(1) The diffuse brain lesions from cysteine vs. the focal lesions from the acidic (excitatory) amino acids including glutamic.

(2) The normal high brain concentration of glutamate vs. the high toxicity of relatively small intakes. This he inferred was a matter of intra- vs. extracellular glutamate, a possible aspect of the blood brain barrier.

He noted the ubiquity both of MSG in foods and of children diagnosed as minimally brain-damaged, inferring a continual hazard to a susceptible minority. He suggested that some susceptibility might be genetic, citing two reports of families, (a) with elevated basal blood glutamate, and (b) with elevated CSF glutamate and normal blood glutamate, each associated with cerebral pathology. He also cited a case of epilepsy that was controlled by avoiding foods containing MSG. He finally reviewed the history of hexachlorophene toxicity and the accident that led to its recognition, suggesting that MSG might be a parallel instance (see also p. 193).

15. In 1969 Pagliara and Goodman (5586) found that fasting plasma glutamate was 40 ± 3 mμmoles/ml in 26 normal adults and 65 ± 4 mμmoles/ml ($P < 0.001$) in 65 patients with gout. Rises induced by casein 0.5 g/kg were considered excessive in the gout group. Plasma glutamine and α-amino nitrogen were normal in both groups, and did not respond to fasting nor to casein. The authors reasoned that glutamate might be a substrate for overproduction of purines in gout (see also p. 98).

V. Glutamate and the Blood-Brain Barrier

1. In 1950 Schwerin et al. (6568) determined the levels of glutamic acid and glutamine in the brain, liver, muscle, and kidney of rats after MSG 1.3 g/kg i.v. (see p. 143). They concluded:

> "It is probable that an inability of the brain to absorb glutamic acid is responsible for the absence of a significant increase in the concentration of either the amino acid or its amide after the administration of glutamic acid. The possibility must be recognized, however, that glutamic acid is absorbed by the brain but is metabolized at such a rapid rate that it does not increase above the normal level."

2. In 1959 Lajtha et al. (4246) demonstrated by a double isotope technique that glutamate was continually exchanged between brain and blood, without increased net uptake by the brain after excessive elevation of the blood glutamate. (For details see pp. 163-168).

3. The experiments of Van Harreveld with various co-workers in 1970-72 (7484,7652,2115) suggested an electrochemical mechanism for the movements of glutamate with respect to the blood-brain barrier. They found that when MSG was injected by microelectrophoresis into the cerebral cortex of an adult rat (4042,7484,7652), an unexpected observation (7652) was the presence of severely

shrunken dark neuronal cell bodies at the injection site, while axons, dendrites, and glia were unaffected or reversibly swollen. Later the cell bodies were removed, so that after two weeks the glutamate spot appeared as a scar devoid of cells. Causation by the injection process was ruled out (7652).

Calculations for the spread of ions so administered, assuming that they were transported by electrical forces through extracellular spaces and removed gradually by cells and blood vessels, were found to predict the tissue changes observed in rat cerebral cortex after the electrophoretic injection of glutamate (7484).

"Glutamate depolarizes neurons when applied electrophoretically. This may cause excitation of the neuronal structures involved, but when pronounced, such a depolarization arrests all activity. If the barrier is permeable for glutamate, excitatory effects as well as a depression of the EEG can, therefore, be expected" (2115). This prediction was verified when newly hatched, surgically prepared chicks were given an adjusted MSG solution i.v. EEG arrest was transient until two days of age, and only the MSG, (not saline nor glucose controls) affected the EEG. On the fifth day, effects of MSG on EEG, impedance, and steady potential were absent, indicating that the barrier had lost its permeablity for glutamate (2115).

In the same report (2115) a retina charged with glutamate-C^{14} was stimulated with unlabeled glutamate, and the label was released. This "regenerative" process was inferred to increase the extracellular glutamate concentration and the permeability of the plasma membrane (2115).

4. In 1972 Perez and Olney (5719) found that in infant mice three hours after s.c. injection of MSG 2 g/kg, there was a fourfold increase in glutamate levels in the arcuate nucleus while in the brain region immediately adjacent,

the ventromedial nucleus of the hypothalamus, and in a more remote region, the lateral nucleus of the thalamus, there were only slight elevations above normal levels (Fig. 4).

Glutamate concentration in the arcuate nucleus was estimated as 10-20 times that in the blood, and the question was why one area, the arcuate nucleus, should progressively concentrate glutamate against a steep gradient while its neurons were being destroyed by the process. Of various possible answers the authors preferred the suggestion, from movements of label (4246 cited, see pp. 163-168), of continual uptake of glutamate by adult brain masked either by exchange or by metabolism. This would be homeostatic for the level of glutamate itself, except in the arcuate nucleus of infant brain.

Earlier, Olney had suggested (5481) an analogous explanation for the retinal concentration of labeled glutamate by the rat, while commenting on a study by Freedman and Potts (2236, see pp. 57-58).

Recently the concept of a blood brain barrier has undergone further modification as a result of three studies demonstrating an effect of the route of administration. These studies are therefore outlined although glutamate was used in only one of the studies.

5. In 1970 Oldendorf (8362) reported regional measurements of brain uptake of C^{14} or S^{35} labeled compounds using H^3-H_2O as an internal reference standard. He injected a mixture of compound and standard into the common carotic artery of a rat with decapitation 15 seconds later. The carbon-tritium ratio in brain tissue relative to that in the injection mixture determined the amount of test substance taken up during a single passage through brain micro-circulation. (See original paper for laboratory details).

The method was demonstrated using sucrose-C^{14} as a nondiffusible test substance and methionine-S^{35} as a diffusible test substance. When 0.015 mM

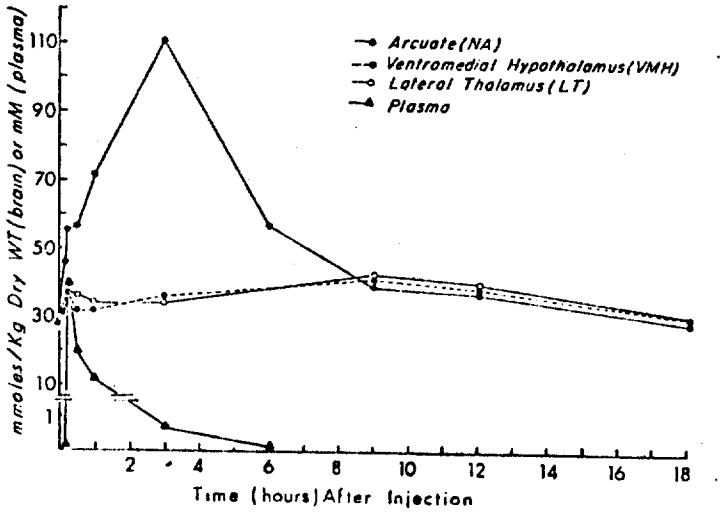


Fig. 4. Blood concentrations and regional distributions of glutamic acid in brains of 4-day-old mice at various times after the subcutaneous injection of monosodium L-glutamate, 2 mg/g body wt. Each point represents the mean of glutamic acid (mmoles/kg dry wt. or mM) for blood from 8 mice or for bilateral samples of NA, VMH and LT from 4 mice at 0 (controls) through 3 hours, 12 mice at 6 hours and 8 mice in each group thereafter. (5719)

methionine was injected, about 34% of available label was taken up by brain, and when 5 mM was injected, about 5.8% was taken up. Tests with 1 μC water alone indicated that about 8% of the injection was distributed to the brain.

6. In 1971 Oldendorf (8363) used the above method (8362) to measure uptake by the brain of 18 amino acids including glutamate.

The results are shown in Table 39. There was a clear separation between uptakes of essential and nonessential amino acids. For this purpose tyrosine was considered essential because of the lack of phenylalanine hydroxylase in rat brain (referenced), and the study demonstrated that rat brain tyrosine was derived from the blood. When glucose-C^{14} was injected, the label appeared rapidly in brain alanine, aspartic and glutamic acids, and to a less extent in glycine and serine, suggesting that these amino acids in the brain were partly derived from blood glucose. The relative uptakes in Table 39 were interpreted as reflecting blood brain barrier permeabilities.

7. In 1971 Dhopeshwarkar et al. (8359) tested a hypothesis that differences of uptake between adult and juvenile brains, ascribed to a barrier, might not survive measurement by Oldendorf's techniques (8362,8363 cited). They used acetate-1-C^{14} as a test substance.

Six Wistar rats, 400 g, sex not stated, fed and watered ad libitum, received 10 μC acetate (45 mC/mM) in 0.2 ml H_2O. Measured brain and liver samples were treated within 45-50 seconds: blood was spun for three minutes, and plasma was quick-frozen. Lipids were extracted, fractionated, quantified, and counted (see paper for details).

Results are summarized in Tables 40, 41 and 42 (see paper for details). The authors commented that whereas previously they had found less fatty acids incorporated into brain lipids than into liver or plasma lipids, they now found the reverse.

Table 39 (8363)

Percentage Uptake of ^{14}C L-Amino Acids and ^{14}C D-Mannitol Relative to $^{3}H_2O$ by Brain After Common Carotid Injection in Rat

	Nutritional Classification	(E) % Taken up by brain	
		Mean	SD (n = 3)
H_2O	---	100	
Phenylalanine	Ess.	54.5	5.4
Leucine	Ess.	51.0	2.7
Tyrosine	Noness.	46.8	3.2
Isoleucine	Ess.	37.3	0.92
Methionine	Ess.	34.5	1.35
Tryptophan	Ess.	33.6	3.8
Histidine	Ess.	31.0	1.8
Arginine	Ess.	20.8	1.9
Valine	Ess.	19.8	2.2
Lysine	Ess.	13.9	2.5
Threonine	Ess.	10.7	0.23
Serine	Noness.	7.05	0.48
Alanine	Noness.	5.50	0.85
Citrulline	Noness.	4.71	1.19
Proline	Noness.	3.05	0.21
Glutamic	Noness.	2.81	0.15
Glycine	Noness.	2.47	0.25
Aspartic	Noness.	2.24	0.57
D-Mannitol	---	1.92	0.23

Relationship between classification of Rose et al. into nutritionally essential or nonessential amino acids in rat and percentage taken up by brain after carotid injection. Amino acid concentration injected was different for each acid depending on the specific activity of the labeled material with a range of 0.008 to 0.05 mM. Radiochemicals were from New England Nuclear, Boston, or from Amersham/Searle, Arlington Heights, Illinois.

Table 40 (8359)

Incorporation of [I-^{14}C]Acetate by Rat Brain, Liver and Plasma Lipids 15 sec After Carotid Arterial Injection

	Spec. act. total lipids (counts/min per mg)	Radioactivity of total lipids (counts/min per g fresh weight of tissue)	% of given dose (μC/100 g of body weight) recovered in total lipids	Cholesterol spec. act. (counts/min per mg)	Polar lipids (phospholipids - sphingolipids) (counts/min per mg)
Blood plasma	168 ± 121	467	0.008	---	---
Brain	326 ± 61	34328	0.64	86	294
Liver	31 ± 25	1241	0.02	105	54

Table 41 (8359)

Specific Radioactivity of Polar Lipids After Administration of [I-^{14}C]Acetate

	Specific activity (counts/min per mg)		
	Brain		Liver
	24 h	15 sec	15 sec
Phosphatidyl choline	130	86	43
Phosphatidyl ethanolamine	75	45	35
Phosphatidyl serine	46	239	5
Sphingomyelin	30	59	15
Ceramid	--	138	--
Cerebroside	51	138	--
Cerebroside sulfate	--	9	--

Table 42 (8359)

Distribution of Radioactivity in Palmitic and Stearic Acid After Injecting [I-^{14}C]Acetate

Dose given	Period between injection of dose and sacrifice of rats	Fatty acid isolated from brain total lipids			
		Palmitic acid		Stearic acid	
		Spec. act. (counts/min per mg)	% RCA	Spec. act. (counts/min per mg)	% RCA
[I-^{14}C]Acetate (i.p.)	24 h	--	14.1	--	20.6
[I-^{14}C]Acetate (i.p.)	4	--	13.5	--	25.6
[I-^{14}C)Acetate (i.c.)	15 sec	595	13.4	91	43.1

i.p., intraperitoneal
i.c., intracarotid
RCA, relative carboxyl activity (radioactivity in -COOH·100/radioactivity in total fatty acids)

They concluded:

"1. There was considerably more uptake of radioactivity into the brain as compared to the liver or the plasma.

2. High radioactivity in the cholesterol fraction indicating rapid synthesis from radioactive acetate.

3. Incorporation of radioactivity into all major polar lipid fractions of the brain lipids including sphingomyelin and cerebrosides, considered to be myelin lipids. Phosphatidyl serine was the most highly labeled component.

4. Palmitic acid isolated from the brain was synthesized de novo from acetate and stearic acid was formed by chain elongation.

All these metabolic reactions occurring so rapidly in the brain are discussed in view of the older concepts that adult brain is a tissue characterized by slow metabolism."

VI. Special Studies

A. Mutagenic

No published studies were found. Two related, unpublished reports were brought to our attention, and are included because no other information on mutagenicity was located. Both studies were performed by the Industrial Bio-Test Laboratories, Inc. for Wm. Underwood Co. in 1973.

Mice

1. In this study (8340) Charles River Strain male albino mice (60-70 days old) were administered a single oral MSG dose via gavage (See Table 43. The treated animals were mated with groups of three untreated females for each of six consecutive weeks. The mating performance is summarized in Table 44. The females were sacrificed one week after removal from the mating cage, approximately

Table 43 (8340)

Test Material: MSG

Mutagenic Study - Albino Mice
Organization of Groups

Group	Dose Level* (g/kg)	Number of Males Treated
C	---	12
T-I	2.7	12
T-II	5.4	12

*MSG was administered as a 27 percent solution in distilled water. Control males received the vehicle in amounts equivalent to those received by the T-II males.

Table 44 (8340)

Test Material: MSG

Mutagenic Study - Albino Mice
Mating Performance

Group	Test Week Number	Number of Surviving Males	Number of Animals Pregnant Number of Females Mated	Mating Index (Percent)
C	1	12	24/36	66.7
	2	12	23/36	63.9
	3	12	26/36	72.2
	4	12	28/36	77.8
	5	12	27/36	75.0
	6	12	29/36	80.6
T-I	1	12	15/36	41.7
	2	12	16/36	44.4
	3	12	14/36	38.9
	4	12	19/36	52.8
	5	12	19/36	52.8
	6	12	21/36	58.3
T-II	1	12	21/36	58.3
	2	12	24/36	66.7
	3	12	24/36	66.7
	4	12	25/36	69.4
	5	12	25/36	69.4
	6	12	29/36	80.6

at mid-pregnancy. The numbers of implantation resorptions and embryos found are summarized in Table 45. Mutation rates were calculated by comparing the mean number of viable embryos in the test group with those in the controls. These calculations are summarized in Table 46. The report concluded that for the two doses of MSG administered, neither embryonic death in utero nor genetic damage, as manifested by dominant lethal mutations, occurred.

2. In the second unpublished mutagenicity study (8339) the host-mediated assay was used to investigate the possible effects of MSG and its metabolites on bacteria indirectly exposed to these compounds. Male Charles River albino mice (250-300 g) were administered MSG at two dose levels by gavage, daily for 14 consecutive days. Twenty-four hours following the last MSG dose, the animals were inoculated i.p. with S. typhimurium. Controls were administered distilled water. One intramuscular injection of dimethylnitrosamine was administered to animals as a positive control (see Table 47 for dose levels).

The bacteria were recovered after remaining in the peritoneal cavity for three hours and the number which had mutated (reverted) to their protrophic form determined. (See the original paper for experimental details and calculation used to determine the mutation rate.) The results of one trial are summarized in Table 48.

The table reporting the results of a second trial was not attached to the report. The researchers concluded from these two trials that in this test system, MSG administered in sub-chronic oral doses was not mutagenic.

B. Teratogenic

1. Landauer (4255) injected 0.05 ml of a solution of 547 mg/ml of L- or DL-glutamic acid hydrochloride dissolved in saline into Leghorn chicken eggs prior to incubation. The increase in frequency of rumplessness, compared with untreated controls, was 3.1 $\pm$ 1.0% for L-glutamic acid hydrochloride and 6.8

Table 45

TEST MATERIAL: MSG

Mutagenic Study - Albino Mice

Summary of Sacrifice Data **(8340)**

Group	Test Week Number	Pregnant Animals Examined	Calculated Corpora Lutea	Implantation Sites	Resorption Sites Early	Resorption Sites Late	Embryos
C	1	24	324	275 (11.4)	12 (0.5)	2 (0.1)	261 (10.9)
	2	23	310	263 (11.4)	8 (0.3)	0 (0.0)	255 (11.1)
	3	26	351	294 (11.3)	13 (0.5)	3 (0.1)	278 (10.7)
	4	28	378	350 (12.5)	10 (0.4)	4 (0.1)	336 (12.0)
	5	27	364	320 (11.8)	18 (0.7)	5 (0.2)	297 (11.0)
	6	29	392	344 (11.9)	15 (0.5)	2 (0.1)	327 (11.3)
T-I	1	15	202	178 (11.9)	6 (0.4)	2 (0.1)	170 (11.3)
	2	16	216	165 (10.3)	7 (0.4)	1 (0.1)	157 (9.8)
	3	14	189	178 (12.7)	5 (0.4)	1 (0.1)	172 (12.3)
	4	19	256	240 (12.6)	10 (0.5)	1 (0.1)	229 (12.0)
	5	19	256	248 (13.0)	5 (0.3)	3 (0.2)	240 (12.6)
	6	21	284	266 (12.7)	18 (0.8)	1 (0.1)	247 (11.8)
T-II	1	21	284	287 (13.7)	13 (0.6)	0 (0.0)	274 (13.0)
	2	24	324	276 (11.5)	6 (0.2)	1 (0.1)	269 (11.2)
	3	24	324	323 (13.4)	11 (0.4)	2 (0.1)	310 (12.9)
	4	25	338	316 (12.6)	11 (0.4)	1 (0.1)	304 (12.2)
	5	25	338	314 (12.6)	10 (0.4)	2 (0.1)	302 (12.1)
	6	29	392	371 (12.8)	25 (0.9)	3 (0.1)	343 (11.8)

Note: Numbers in parentheses are mean values.

Table 46

TEST MATERIAL: MSG

Mutagenic Study - Albino Mice

Summary of Mutagenic Data **(8340)**

Group	Test Week Number	Pre-Implantation Loss (Percent)	Mutation Rates A	B a	B b
C	1	15.1	4.4	-	5.2
	2	15.2	3.0	-	2.6
	3	16.2	4.4	-	7.0
	4	7.4	2.8	-	-4.3
	5	12.1	5.6	-	5.2
	6	12.2	4.4	-	2.6
T-I	1	11.9	3.4	-3.7	1.7
	2	23.6	4.2	11.7	14.0
	3	5.8	2.8	-15.0	-7.0
	4	6.2	4.2	0.0	-4.3
	5	3.1	2.0	-14.5	-8.6
	6	6.3	6.8	-4.4	-1.7
T-II	1	0.0	4.5	-19.3	-13.0
	2	14.8	2.2	-0.9	2.6
	3	0.3	3.4	-20.6	-12.2
	4	6.5	3.5	-1.7	-6.1
	5	7.1	3.2	-10.0	-4.3
	6	5.4	6.7	-4.4	-1.7

Table 47

Test Material: MSG

Host-Mediated Mutagenic Study - Albino Rats
Organization of Groups (8340)

Group	MSG Dose Level (g/kg)	DMN Dose Level (mg/kg)	Number of Animals Trial 1	Trial 2
C	0	0	4	4
PC	0	100	4	4
T-I	0.2	0	4	4
T-II	5.7	0	4	4

Note: In order to ensure recovery of bacteria from at least 3 animals per group, 4 animals from each group were inoculated at each trial. DMN (dimethylnitrosoamine) was administered as a single intramuscular injection at the time of inoculation.

Table 48

Test Material: MSG

Host-Mediated Mutagenic Study - Albino Rats
Normal and Revertant Bacteria Counts (8340)

Group	Animal Number	Organisms per ml of Aspirate Total x 10^6	Revertants	Mutation Rate
C	1	330.00	8.75	2.65
	2	310.00	8.75	2.82
	3	327.50	6.25	1.91
	Average			2.45
PC	1	345.00	22.50	6.52
	2	350.00	21.25	6.07
	3	432.50	46.25	10.69
	Average			7.98
T-I	1	302.50	7.50	2.48
	2	327.50	6.25	1.91
	3	315.00	5.00	1.59
	Average			1.98
T-II	1	335.00	12.50	3.73
	2	320.00	3.75	1.17
	3	327.50	2.50	0.76
	Average			1.91

± 1.3% for the DL-glutamic acid hydrochloride. According to the author, the data suggested that the DL-isomer produced a greater frequency of rumplessness than the L-form. He found these compounds produced no significant increase in the incidence of other morphological abnormalities.

In a later experiment Landauer and Rhodes (4254) injected L-glutamic acid hydrochloride (12.5 and 25 mg per egg) into chicken eggs. At 24 hours the L-glutamic acid hydrochloride was somewhat toxic and was markedly so at 96 hours. Mortality for 24-hour embryos was 19.5 ± 3.45% during the first six days. There was a slight tendency to rumplessness (6.5 ± 2.46%).

2. Aleksandrov et al. (0168) injected intravitellinely, among other compounds, DL-glutamic acid (1 mg in 0.1 ml physiological solution) into 43 fertilized chicken eggs prior to incubation. Developmental defects were observed in brain (7), eye (7), beak (4), abdominal wall (4), extremities (5), and body (4). Twenty-five percent of the eggs injected with DL-glutamic acid had abnormalities of development, compared with 3.6% of the controls.

3. Tugrul in 1965 (7542) gave three groups of rabbits 25 mg/kg of glutamic acid for 40 days (D- or L- form not specified). In group 1 (10 females, 4 males) the females only received glutamic acid; in the second group (4 females, 2 males) both sexes received glutamic acid in combination with vitamin B_6; in the third group (6 females, 2 males) glutamidine, a pharmaceutical based on the hydrochloride of glutamic acid, was given. All doses were given orally.

At sacrifice, autopsy of group 1 showed two rabbits with arrested pregnancy, calcareous deposits in the region corresponding to the endometrium, and a degenerated fetus, and two females with postabortive endometritis of the uterus. Two rabbits had term deliveries but the offspring had various malformations, according to the author. Hyperplastic uteri were found in two rabbits which

did not deliver. The offspring of those that did deliver did not become pregnant and developed crippling deformities of the extremities and spinal curvature. Growth and development of offspring were sharply retarded, compared with controls, and tissue studies revealed atrophy of the adipose tissue, endometrium, testes with cessation of spermatogenesis, hyperplastic adrenal capsules, and increases of basophilic cells in the anterior pituitary.

In the second group, no abortions were observed, but two rabbits gave birth to offspring with deformed extremities, three of which died shortly after birth. The parents again showed hyperplastic uteri and inhibition of spermatogenesis. The offspring displayed the same gross and histological abnormalities found in group 1. Similar results were obtained with the third group.

Nerve tissue damage was observed only as a hyperemia, which was attributed to inhibition of thrombokinase by glutamate. Atrophy of adipocytes in offspring was attributed to metabolic results of an affinity of glutamate for liver lipids and serum lipoproteins.

4. A Japanese group giving the same dosage of glutamic acid hydrochloride and MSG (D- or L- form not specified) to pregnant rabbits for 15 days found a higher incidence of gestational abnormalities in their controls which were fed saline solution (concentration not specified) than in the experimental group (Ajinomoto Co., unpublished report). The authors noted that if MSG were added to strongly acidic or alkaline foods that were then heated to 100°C or more, it could undergo partial racemization to the D-form (Ajinomoto, unpublished company report).

C. Therapy

While therapeutic applications of the glutamates may not be directly relevant to their safety as food additives, some of the major clinical

uses of glutamic acid are summarized because of possible relevance to the metabolic behavior of glutamic acid in the human body.

There have been two main areas in which glutamic acid was used therapeutically: in hepatic coma and in attempts to improve the intelligence and behavior of mentally retarded persons.

Krebs (4044 cited in 6399) had found in 1936 that glutamic acid caused a reduction of ammonium ion concentration in brain tissue slices. Sapirstein (6399) hypothesized that since ammonium convulsions closely simulated spontaneous seizures, glutamic acid might prevent other seizures also. He found that rabbits with ammonium-induced convulsions were protected by i.v. injections of glutamic acid. Subsequently, infusions of glutamic acid were used to treat cases of hepatic coma, but Pearlman (5685) found that while glutamic acid did decrease ammonia levels in patients with hepatic dysfunction or hepatic coma, it was of permanent value only in cases in which the liver retained some capacity to recompensate. Iber et al. (3185) attempted to resolve the conflicting accounts of glutamate's clinical effectiveness in this area; they gave glutamic acid infusions to two normal subjects and eight patients with cirrhosis and found that the pattern of response was similar for normal subjects, patients with cirrhosis, and patients with hepatic coma. Blood ammonia levels fell in 11 of 12 administrations, α-ketoglutarate levels rose in 8 of 10 instances, and changes in blood pyruvate and citrate were variable. Glutamine levels did not rise sufficiently in all cases to account for the lowering of blood ammonia, and only 1 of 7 patients in hepatic coma had a clinical response to sodium glutamate infusion.

The widespread clinical use of i.v. doses of glutamic acid led Mazurowa et al. (4761) in 1962 to test the effect of glutamic acid on the circulatory system of rabbits. Large doses of glutamic acid given i.v. to rabbits

produced EKG changes that suggested myocardial lesions. Doses of 15% MSG above 2.5 ml produced arrhythmia and cardiac arrest in the isolated cat heart, along with coronary constriction. These results did not occur when the drug was given orally. Glutamate-induced arterial hypotension in isolated cat heart was found to be of central origin. The authors warned against indiscriminate use of glutamic acid, especially in i.v. injections (4761).

In 1943 Price et al. (5920) reported that eight patients subject to petit mal seizures seemed brighter when they received DL-glutamic acid hydrochloride to control their convulsions. Grand mal seizures were unaffected. The authors cautioned that their observations were preliminary and required confirmation (5920).

In 1960 Astin and Ross (0366) reviewed the use of glutamate as a medication for mental deficiency and concluded that positive responses tended to be reported in studies not using a control group. They found that the few positive controlled studies contained methodological flaws. Negative findings tended to occur in adequately designed studies (0366).

However, this conclusion of Astin and Ross was challenged six years later in the same journal by Vogel et al. (7738), and in 1969 Wincze and Vogel attempted to account for discrepant findings in the learning ability of rats (8038). They gave 30-day-old Sprague-Dawley rats 50-400 mg of L-glutamic acid daily in applesauce along with a standard lab chow for 60 days. The 200-mg glutamic acid group displayed consistent superiority at bar-pressing while the 400-mg animals were hyperemotional, a trait also associated with toxic levels of glutamic acid supplementation in human subjects. Wincze and Vogel concluded that moderate levels of glutamic acid improved performance on simple perceptual motor tasks but high levels led to overactivation and behavioral disorganization. They commented that as a sympathetic stimulant glutamate should not be expected to benefit perceptual-restructuring performances, e.g., maze-running.

Weiss et al. (7915) fed weanling rats 10% MSG, MKG, NaCl, or KCl for 34 weeks and found that motor activity was reduced in MKG and KCl rats compared to controls, MSG, and NaCl rats. Animals receiving MSG failed to develop avoidance lever-pressing responses, but rats receiving MKG or KCl showed a high rate of avoidance responses.

C. Carcinogenic

Only one published study relevant to the glutamates was found.

In 1972 Greenberg (2654) fed Sprague-Dawley rats diets with large amounts of MSG for long periods and explored the vascular and reticuloendothelial systems for effects (no details given).

An early increase of basket cells in peripheral blood was followed, after several weeks, by lymphoid cells fitting the description "neoplastic." Also, abnormal neutrophils were seen that resembled those described in some cases of granulocytic leukemia.

Nevertheless the author stated that he interpreted these findings, not as leukemia, but as effects of endothelial injury resulting in fragile, degenerated phagocytes.

BIOCHEMICAL ASPECTS

I. Breakdown

The breakdown of the various glutamates in the body is discussed in the following sections; breakdown in foods is discussed briefly in Chemical Section VIII. No breakdown in storage was noted.

II. Absorption

A number of experiments have been made to correlate blood levels with oral or injected doses of MSG.

A. Mice

Schwerin et al. (6568) injected into the tail vein of mice (16-18 g, strain and sex not given) a 7% (1.2-1.3 mg/g BW) aqueous solution of MSG. The animals were sacrificed after various time intervals (see Table 49) and the glutamate concentrations in brain, liver, muscle, kidney, and blood were recorded. The amount of MSG injected was expected to produce initial blood levels of 1.0-1.5%, but 10-15 minutes after administration only a small fraction of the dose could be accounted for in the blood levels.

The concentrations of glutamate in the liver, muscle, and kidney were increased after administration of MSG but there was no significant increase in the brain concentration. The authors suggested that:

(1) There was no increase observed in brain glutamate level because it was metabolized rapidly after absorption. Alternatively --

(2) If there was any uptake by the gray matter it could not be detected since the analyses were of the whole brain (see p. 123).

B. Rats

Similar treatment given to rats (160-180 g) (see Table 50), produced

Table 49 (6568)

Glutamate Concentrations in Mouse Organs After Intravenous Injection of Glutamic Acid (as the Sodium Salt)

	Acid			
Min.	Brain	Liver	Kidney	Blood
0[a]	169±14[b]	19	78	3.1±0.6
15	153	70		
15	171			45
15	204		574	172
15	178			153
30	195			14
30	184			98
30	166			32
45	134			38

[a]The number of groups of three animals represented by the averages were brain (five), liver (one), kidney (one), and blood (three).

[b]Standard deviation.

Table 50 (6568)

Glutamate Concentration in Rat Organs After Intravenous Injection of Glutamic Acid (as the Sodium Salt)[a]

Min.	Brain	Liver	Muscle	Kidney	Blood
0	152±16	49±14	18±2.6	96±4.5	3±0.7
10	120	94	40	-	-
10	144	99	86	400	109
15	122	63	18	490	63
15	111	111	54	520	90
20	140	72	--	-	-
20	158	114	--	424	85
30	116	-	--	510	68
60	131	-	--	260	4.6

[a]Values expressed as mg/100 g.

similar results (6568).

C. Rabbits

Neame and Wiseman (5238) investigated the absorption of a neutralized 2% L-glutamic acid solution (6-10 ml/kg BW) inserted daily into the intestines of four rabbits (1.6-3.0 kg). Table 51 shows the results:

(1) An average 49% of the glutamic acid disappeared from the lumen after 30 minutes.

(2) Because blood from the small intestine of the rabbit was diluted by blood from the large intestine, the latter was removed so as to demonstrate an increase in alanine concentration in the mesenteric venous blood as it passed through the small intestine during absorption and transamination of glutamic acid.

(3) The absorption and transamination of glutamic acid in the intestine did not affect the mesenteric blood concentrations of pyruvate and α-oxoglutarate.

D. Cats

1. Whether glutamic acid was converted to glutamine was studied by Bessman et al. (0721) who injected 100 mg of glutamic acid into the small intestine of two cats. After 15 minutes, a portal blood sample indicated absorption of glutamic acid without conversion to glutamine (glutamine concentration decreased in a 15-minute interval). A subsequent (at 30 minutes) marked increase in glutamine concentration was attributed to its release from the tissues.

2. When Neame and Wiseman (5238) introduced 2% L-glutamic acid solution (6-10 ml/kg BW) into the intestines of 3 cats (1.6-3.0 kg) there was an increase of alanine (see Table 52) in the mesenteric blood as it passed through the small intestine, indicating that most of the glutamic acid was transaminated

Table 51 (5238)

Alanine, Pyruvate, and α-Oxoglutarate Concentrations in Arterial (Art.) and Mesenteric Venous (Mes.) Blood taken 30 min. After the Introduction of 2% L-Glutamic Acid Solution into the Lumen of the Small Intestine of the Rabbit[a]

	Alanine (μmole/100 ml)		Pyruvate (μmole/100 ml)		α-Oxoglutarate (μmole/100 ml)		Glutamic acid absorbed		
Expt. no.	Art.	Mes.	Art.	Mes.	Art.	Mes.	(μmole)	(μmole/kg body wt)	% of amount introduced
1	104	153	36	30	10	11	935	580	42
2	217	286	87	57	9	6	2350	940	67
3	208	297	45	25	9	10	1910	710	60
4	153	230	67	41	7	13	1460	660	50
Control 1	127	124	76	53	4	3	--	--	--
Control 2	125	114	40	32	4	4	--	--	--

[a]Only small intestine in situ. In the control experiments the small intestine was left empty.

Table 52 (5238)

Alanine, Pyruvate, and α-Oxoglutarate Concentrations in Arterial (Art.) and Mesenteric Venous (Mes.) Blood taken 30 min. After the Introduction of 2% L-Glutamic Acid Solution into the Lumen of the Small Intestine of the Cat[a]

	Alanine (μmole/100 ml)		Pyruvate (μmole/100 ml)		α-Oxoglutarate (μmole/100 ml)		Glutamic acid absorbed		
Expt. no.	Art.	Mes.	Art.	Mes.	Art.	Mes.	(μmole)	(μmole/kg body wt)	% of amount introduced
1	78	124	19	26	5	5	1403	830	94
2	108	169	21	24	2	2	2320	800	86
3	25	51	10	11	1	1	1706	1070	95
Control 1	47	40	14	12	1	1	—	—	—
Control 2	64	63	15	16	1	2	—	—	—

[a]Small and large intestine in situ. In the control experiments the small intestine was left empty.

before it reached the blood. The concentrations of pyruvate and α-oxoglutarate in the blood passing through the intestine were unchanged.

E. Dogs

Neame and Wiseman (5235 and 5238) showed that in dogs (12-20 kg), during the absorption of glutamic acid, the concentration of alanine increased in the venous blood draining the area of the intestine studied. This increase depended on the concentration of glutamic acid in the intestinal lumen. When a 10% glutamic acid solution was used, most of it was absorbed as glutamic acid (see Table 53). When solutions of lower concentration were used, most of the glutamic acid was transaminated before reaching the blood, and alanine concentrations showed a marked increase in the mesenteric blood (see Tables 54 through 57).

F. Pigs

In 1973 Steginк et al. (8364) intubated fasted 3-day-old piglets with MSG 0.01, 0.1, and 1.0 g/kg in water or infant formula, doses intended to cover the range and tenfold the range of human infant exposures. [But see pp. 199-202]. Samples of plasma, spinal fluid, muscle, and brain were analyzed for amino acids.

Plasma glutamate peaks occurred at 20 minutes when 1 g/kg MSG was given in water, and 90-120 minutes when it was given in formula (see Fig. 5 for typical data). The 0.01 g/kg dose produced no significant differences from controls, and the 0.1 g/kg dose only minor differences at peak absorption times. MSG 1 g/kg also produced significant rises of plasma alanine and aspartate, but not glutamine. In fasted piglets portal plasma glutamate was five-fold the level in peripheral plasma, but the other parameters were not significantly different. Free amino acids did not change significantly in spinal fluid,

Table 53 (5235)

Blood Glutamic Acid and Alanine Concentrations After Introduction of 10% Glutamic Acid Solution into Intestinal Lumen[a]

Sample	Collected at (min)	Volume (ml)	Glutamic acid conc. (μmole/100 ml)	Alanine conc. (μmole/100 ml)
Arterial	5	25	104	35
	19	25	103	41
	42	25	127	49
	67	25	137	47
	95	25	150	95
Venous	0-5	30	60	43
	5-16	60	108	44
	16-27	60	205	68
	27-40	65	246	76
	40-53	60	311	124
	53-73	60	570	157
	73-99	63	680	190

[a]In all tables arterial samples from carotid artery; venous samples from vein draining experimental portion of small intestine. Experimental solutions introduced into intestinal lumen after collection of first venous sample.

Table 54 (5235)

Blood Glutamic Acid and Alanine Concentrations After Introduction of 0.15% Glutamic Acid Solution into Intestinal Lumen

Sample	Collected at (min)	Volume (ml)	Glutamic acid conc. (μmole/100 ml)	Alanine conc. (μmole/100 ml)
Arterial	2	25	120	110
	51	25	102	123
	90	25	127	197
Venous	0-8	30	99	119
	8-17	30	99	112
	17-26	30	99	139
	26-36	30	98	153
	36-45	30	--	--
	45-55	30	85	153
	55-64	30	69	182
	64-76	30	87	177
	76-90	30	76	182

Table 55 (5235)

Blood Glutamic Acid and Alanine Concentrations After Introduction of 0.5% Glutamic Acid Solution into Intestinal Lumen

Sample	Collected at (min)	Volume (ml)	Glutamic acid conc. (μmole/100 ml)	Alanine conc. (μmole/100 ml)
Arterial	4	25	87	59
	65	25	92	91
	91	25	102	136
Venous	0-8	30	84	73
	8-21	30	102	103
	21-34	30	186	169
	34-48	30	161	160
	48-65	30	145	186
	65-95	30	130	189

Table 56 (5235)

Blood Glutamic Acid and Alanine Concentrations After Introduction of 2% Glutamic Acid Solution into Intestinal Lumen

Sample	Collected at (min)	Volume (ml)	Glutamic acid conc. (μmole/100 ml)	Alanine conc. (μmole/100 ml)
Arterial	5	25	82	71
	31	25	86	74
	73	25	77	92
	101	25	84	97
Venous	0-7	30	62	121
	7-15	30	65	119
	15-23	30	212	147
	23-30	30	435	159
	30-37	30	460	165
	37-49	30	489	204
	49-59	39	420	217
	59-71	30	409	216
	71-85	30	359	202
	85-102	30	265	229

Table 57 (5235)

Alanine, Pyruvate, and α-Oxoglutarate Concentrations in Arterial (Art.) and Mesenteric Venous (Mes.) Blood taken 30 min. After the Introduction of 2% L-Glutamic Acid Solution into the Lumen of the Small Intestine of the Dog[a]

	Alanine (μmole/100 ml)		Pyruvate (μmole/100 ml)		α-Oxoglutarate (μmole/100 ml)		Glutamic acid absorbed		
Expt. no.	Art.	Mes.	Art.	Mes.	Art.	Mes.	(μmole)	(μmole/kg body wt)	% of amount introduced
1	133	151	43	41	0	0	4649	580	87
2	56	79	22	22	4	5	11430	820	65
3	79	113	23	23	2	1	9250	840	69
Control 1	75	66	41	37	1	1	--	--	--
Control 2	42	46	9	9	0	0	--	--	--

[a]Small and large intestine in situ. In the control experiments the small intestine was left empty.

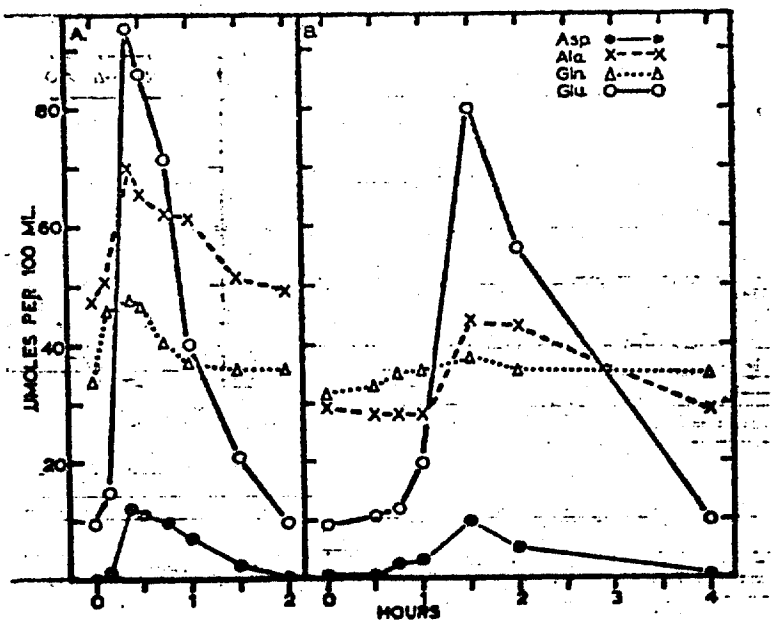


Fig. 5. Plasma amino acid levels in a typical neonatal pig following an oral load of 1 g/kg MSG administered in water (A) and in infant formula (B). Blood samples obtained from the vena cava. (8364)

brain, or muscle after the two lower doses; data from the 1 g/kg dose were not reported.

The authors commented that plasma glutamate levels rose only when the immediate capacity of the liver to metabolize it was exceeded. The changes after MSG 0.1 g/kg were similar to those found by the authors in lactating women (cited). Piglet brain samples (hypothalamus) were obtained 90-150 minutes after oral loading, which according to the authors might not have been the best time interval. In review, they also emphasized the importance of the route of administration.

G. Humans

1. When four normal subjects were given glutamic acid orally (1 g/10 kg BW) by Bessman et al. (0721), there were increases of various amounts in the concentrations of glutamic acid in the peripheral blood (see Table 58). After one hour, two subjects showed a slight increase in glutamic acid blood levels and an increase in glutamine levels, while the other two subjects showed a greater increase in glutamic acid levels and a reduction in glutamine levels. The authors noted that their findings with both humans and cats (see p. 146) which included both decreases and increases of portal blood glutamine concentration, demonstrated the influence of the blood level of one amino acid on that of another.

Both these authors and Waelsch (7787) concluded that the ratio of glutamine to glutamic acid in protein might influence the relative glutamic acid intake when only a small supplement was ingested. Waelsch (7787) estimated that ingesting an additional 10 g of glutamic acid with 100 g of food already containing 10 g each of glutamic acid and glutamine doubled the actual intake of the acid. They also noted that the experiments (cat and human) did not

Table 58 (5235)

Concentration of Free Glutamic Acid and Glutamine After Oral Administration of Glutamic Acid to Human Subjects (1 g/10 kg)[a]

Time after administration (hr)	Subject 1		Subject 2		Subject 3		Subject 4	
	Acid	Amide	Acid	Amide	Acid	Amide	Acid	Amide
0	0.6	8.4	0.6	10.6	1.2	8.4	0.8	10.3
1	1.0	10.3	1.2	14.0	5.0	5.8	9.5	8.3
2	1.0	8.9	0.8	11.3	0.7	6.3	1.0	10.0

[a]Values expressed as mg/100 ml of plasma.

predict what the glutamic acid and glutamine blood levels would be if glutamic acid were ingested in combination with other amino acids, as in protein hydrolysates.

2. Himwich (2996) studied in adult humans the rates of absorption of the three forms of L-glutamic acid usually administered: the sodium or potassium salt, the unneutralized acid, and the hydrochloride. It was postulated that since the solubilities of these three compounds differed markedly (L-glutamic acid, 0.7 g/100 g water, 20°C; L-glutamic acid hydrochloride, 33.3 g/100 ml water, room temp.; MSG, 73.9 g/100 g water, 25°C), so probably would their absorptions. Fifteen grams of each of the compounds in tomato juice and water were ingested by human subjects. Blood samples before ingestion and at half-hour intervals up to three hours after ingestion were taken (along with four cases at four and five hours). See Fig. 6 for tolerance curves for one of the subjects. A wide variation of absorptions was found: in three cases the administration of the free acid with food slightly raised its absorption, but not to a level comparable to the salt; one case showed no rise in blood level whether the acid was ingested with or without food; only one case showed a delayed rise; only two cases showed a pattern similar to that reported by Bessman et al. (0721).

The author concluded that the unneutralized acid was probably not absorbed to any great extent, but passed through the gut without going into solution. The unpleasant effects of the hydrochloride given in solution (nausea, stomach cramps, marked fall in blood pressure), as well as the normal pattern of variation of the plasma glutamate level after ingestion of the unneutralized acid, led the author to suggest that much of the material actually administered in glutamic acid experiments was in the form of the sodium salt, since

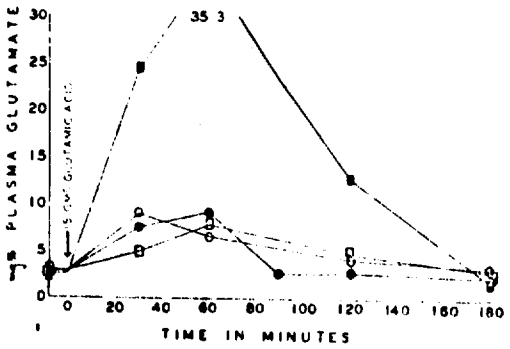


Fig. 6. L-Glutamic acid tolerance curves on one subject with L-glutamic acid compounds equivalent 15 g of the acid. ■ Neutralized. □ Hydrochloride. ● Unneutralized. ○ Unneutralized with food. (2996)

many investigators failed to specify the form they had used. This could account for much confusion found in the literature concerning the activity of L-glutamic acid. (2996)

3. In 1972 Stegink et al. (8354) investigated the effect of the oral administration of MSG on free amino acid levels in plasma and breast milk. A dose of MSG equivalent to 0.1 g/kg BW (6g) was orally administered to ten lactating women in either water or Slender. Four control subjects were given 6g lactose in conjunction with water. Milk samples, urine samples, and blood samples were obtained at various intervals after administration of either MSG or lactose. The changes noted in the plasma amino acid levels for four acids are summarized in Table 59. Free amino acid levels in milk were found to vary considerably among the individuals tested and among sampling times. These levels are summarized in Table 60. The authors found small increases in plasma glutamate, aspartate, and alanine levels. Little change in breast milk amino acid levels was observed. They concluded that little if any of the administered MSG was concentrated in the milk.

III. Metabolism and Excretion

The concentration of glutamic acid is disproportionately high in the brain compared with the majority of free amino acids (7895). Brain differs from muscle and other tissues in that it contains enzyme systems for amidation and decarboxylation of glutamic acid as well as the transamination and oxidation systems common to the other tissues (7895).

According to Walshe (7840), glutamic acid is the only amino acid oxidized by the brain. It is associated with carbohydrate metabolism via deamination to α-ketoglutaric acid, which then enters the Krebs cycle. It also forms glutamine by binding ammonia which is not liberated when glutamine is metabolized.

Table 59. Plasma Amino Acid Levels (8354)

Time (min)	Amino acid (μmoles/100 ml plasma)			
	Aspartate	Glutamine	Glutamate	Alanine
MSG with water ($N = 4$)				
0	0.32 ± 0.16	61.9 ± 16.8	3.90 ± 1.70	53.1 ± 13.3
30	1.04 ± 0.92	68.0 ± 7.10	13.0 ± 10.1	50.6 ± 11.4
60	0.54 ± 0.31	62.4 ± 5.80	7.5 ± 4.80	46.6 ± 5.60
120	0.70 ± 0.40	65.9 ± 14.9	5.10 ± 2.70	47.3 ± 10.5
180	0.45 ± 0.25	62.4 ± 8.07	4.75 ± 2.31	40.5 ± 10.5
MSG with Slender ($N = 9$)				
0	0.64 ± 0.27	61.0 ± 3.70	4.34 ± 0.70	42.5 ± 6.32
60	1.28 ± 1.42	70.5 ± 14.7	7.05 ± 2.70	68.1 ± 19.6
90	1.27 ± 1.24	72.1 ± 14.0	9.23 ± 5.34	67.3 ± 12.1
150	1.84 ± 1.45	65.0 ± 10.4	11.8 ± 8.20	50.3 ± 10.4
210	1.32 ± 0.80	59.6 ± 8.31	10.2 ± 7.99	46.8 ± 6.95
Lactose with water ($N = 4$)				
0	0.43 ± 0.14	69.7 ± 16.6	4.00 ± 0.97	40.5 ± 6.60
30	0.65 ± 0.07	83.6 ± 8.70	4.10 ± 0.28	48.3 ± 6.70
60	0.39 ± 0.10	76.4 ± 4.60	3.70 ± 1.40	46.3 ± 10.0
90	0.56 ± 0.26	65.0 ± 16.6	3.80 ± 1.90	41.7 ± 11.4
150	0.61 ± 0.26	71.2 ± 10.9	5.70 ± 4.40	35.1 ± 3.50
210	0.26 ± 0.10	63.0 ± 14.1	3.20 ± 0.56	35.9 ± 4.00

Table 60. Free Amino Acid Levels in Breast Milk Following Administration of MSG or Lactose (8354)

Amino acid	Time following administration (hr) 0	1	2	3	4	6	12
				(μmoles/100 ml milk)			
				MSG with water ($N = 4$)			
Aspartate	2.9 ± 1.0	3.0 ± 3.0	8.0 ± 3.4	8.1 ± 3.9	7.9 ± 2.5	7.5 ± 3.1	9.1 ± 2.1
Glutamine	34.3 ± 19.1	35.8 ± 19.8	55.1 ± 20.3	52.5 ± 18.0	53.9 ± 7.7	51.0 ± 11.9	47.3 ± 12.7
Glutamate	197 ± 61.0	113 ± 27.3	126 ± 17.1	153 ± 66.7	145 ± 16.4	157 ± 36.0	181 ± 32.0
Alanine	16.5 ± 6.3	22.4 ± 6.8	30.1 ± 6.6	29.8 ± 7.5	31.0 ± 5.6	35.0 ± 6.1	35.0 ± 3.7
				MSG with Slender ($N = 9$)			
Aspartate	2.5 ± 1.3	2.9 ± 1.6	5.3 ± 1.7	7.9 ± 2.7	8.0 ± 4.3	10.3 ± 1.5	5.8 ± 2.9
Glutamine	51.8 ± 27.2	42.4 ± 16.2	44.5 ± 12.7	57.0 ± 19.8	53.4 ± 22.4	88.1 ± 27.5	57.0 ± 23.0
Glutamate	146 ± 51.1	118 ± 39.7	128 ± 50.4	150 ± 34.1	161 ± 53.8	182 ± 28.0	159 ± 32.3
Alanine	16.0 ± 6.0	16.4 ± 4.6	25.8 ± 9.1	29.6 ± 9.8	27.4 ± 10.3	33.0 ± 7.8	29.2 ± 10.8
				Lactose with water ($N = 4$)			
Aspartate	2.60 ± 1.6	3.0 ± 0.5	4.0 ± 1.6	4.3 ± 0.8	4.4 ± 4.5	4.2 ± 2.0	4.5 ± 2.0
Glutamine	79.5 ± 44.5	79.9 ± 55.1	68.7 ± 35.5	62.6 ± 34.0	67.3 ± 37.2	70.6 ± 38.0	85.1 ± 34.1
Glutamate	128 ± 36.6	147 ± 27.1	145 ± 16.2	167 ± 51.0	175 ± 49.2	158.7 ± 80.5	181 ± 34.1
Alanine	17.5 ± 7.4	22.7 ± 6.1	23.4 ± 5.2	23.4 ± 5.3	20.9 ± 10.9	26.1 ± 12.0	33.1 ± 10.4

In this way it acts to remove intracellular ammonia from the tissue. Glutamic acid is also important for acetylcholine synthesis and cation transport in the brain and kidney (7840).

A. Mice

In 1959 Lajtha et al. (4246) studied the in vivo metabolism of glutamic acid in the brain by the penetration of ^{14}C-labeled glutamic acid into the brain as well as its conversion to glutamine, γ-aminobutyric acid (GABA), and glutathione (GSH) in brain, liver, kidney, and muscle.

Adult mice (about 100 days old) were injected in the tail vein with ^{14}C-glutamic acid (0.5 μC and 7 mg% glutamic acid). The bulk of the labeled glutamic acid entered the organs rapidly in the form of the acid without first being converted to glutamine. Table 61 summarizes the results. In a few minutes the glutamic acid, glutamine, and GSH fraction of the brain, liver and kidney as well as the GABA fraction of the brain were significantly labeled. Experiments of longer duration supported the evidence for the rapid metabolism of glutamic acid and its metabolic derivatives (see Table 62). (See also p. 123).

B. Rats

1. Lajtha et al. (4246) also performed similar experiments with adult Sprague-Dawley rats (about 100 days old) with essentially similar results and conclusions. Tables 63 and 64 summarize the results. In 1961 the same workers concluded (0682):

(1) In intact animals there was a very rapid exchange of plasma glutamic acid with the free glutamic acid of the brain and the other organs.

(2) Glutamic acid could enter or leave the organs without conversion to glutamine.

(3) The following reactions were shown to occur within 2 minutes after

Table 61 (4246)

Specific Activity of Compounds After Injection of ^{14}C Glutamic Acid

Duration of experiment (min)	Organ	Specific activity of compounds (counts/min per μmole)			
		Glutamic acid	Glutamine	γ-Amino-butyric acid	Glutathione
2	Plasma	45,000	5200		
	Brain	85	57	31	25
	Liver	2800	2100		150
2	Plasma	12,000	1900		
	Brain	15	13	3.4	8.3
	Liver	1100	510		38
	Red blood cells	1900	220		65
	Kidney	704	390		340
3	Plasma	8300	4500		
	Brain	620	160	20	
	Liver	12,000	20,000		
5	Plasma	6000	1900		
	Brain	90	36	33	
	Liver	1200	850		
5	Plasma	22,000	4100		
	Brain	95	78	34	51
	Liver	2400	2000		200
10	Plasma	8000	3000		
	Brain	920	230	60	
	Liver	9200	21,000		

Four adult mice were pooled for each point. They were injected with ^{14}C glutamic acid intravenously. 0.5 μC and 7 mg% glutamic acid was injected in each animal corresponding to about 30% of the plasma glutamic acid content and 4% of blood volume. All values were corrected for counts introduced by blood remaining in the excised organ

Table 62 (4246)

Distribution of Radioactivity In Vivo After Longer Experimental Periods

Duration of experiment (hr)[a]	Organ	Specific activities (counts/min per μmole)		
		Glutamic acid	Glutamine	γ-Aminobutyric acid
2	Plasma	1600	400	36
	Brain	61	47	
	Liver	92	100	
5	Plasma	2000	890	10
	Brain	12	21	
	Liver	34	92	
19	Plasma	2000	1300	(2)
	Brain	20	4	
	Liver	22	80	

[a]Each time point is the average of two experiments. For experimental conditions see legends for Table 42.

Table 63 (4246)

Distribution of Radioactivity in Rat Organs 5 min. After Administration of ^{14}C Glutamic Acid

Organs	Experiment 1			Experiment 2		
	Glutamic acid	Glutamine	GSH	Glutamic acid	Glutamine	GSH
Plasma				3200	670	
Brain	32	27	18	43	17	16
Liver	800	530	110	1800	460	130
Kidney				860	470	560
Muscle				400	46	45

Two rats in each experiment received i.v. (tail vein) 0.1 ml of a solution containing 5 μC/ml uniformly labeled ^{14}C-glutamic acid. Each animal received 11 mg% glutamic acid comprising 7% of the glutamic acid circulating in its plasma. All values are corrected for counts introduced by blood remaining in the excised organ. Values expressed as counts/minute per μmole.

Table 64 (4246)

Total Radioactivity in Whole Rat Organs[a]

	Experiment 1			Experiment 2		
	Glutamic acid	Glutamine	GSH	Glutamic acid	Glutamine	GSH
Plasma[b]				6400	4700	
Brain	640	220	81	860	140	72
Liver	18,000	20,000	6900	40,000	17,000	8200
Kidney				14,000	1600	4000
Muscle[b]				30,000	12,000	4700

[a]Derived from Table 41; expressed as counts/min in the whole organ.
[b]Estimated total weight.

i.v. injection of ^{14}C-glutamic acid:

(a) decarboxylation of glutamic acid to GABA in brain,

(b) interconversion of glutamic acid and glutamine,

(c) incorporation of glutamic acid into GSH in brain and liver.

(4) Within the same 2-minute period the specific activity of brain GABA rose to 40% of the specific activity of glutamic acid in the brain, and that of glutamine to 60%.

(5) In short-time experiments the specific activity of plasma glutamine was generally higher than that of organ glutamine or glutamic acid, when glutamic acid was injected i.v. This finding suggested that glutamine synthesis was compartmentalized (0682).

2. In 1957 Birnbaum et al. (8324) studied the growth response when various sources of "non-essential" nitrogen were added to the basal diet of weanling rats. Each dietary study used six or more male Sprague-Dawley weanling rats. To establish a growth base-line a basal diet, which consisted of the ten "essential" amino acids to 9.5 g total N plus additional components in 50% aqueous solution, was first offered ad libitum for 21 days to six weanling rats. Next, various amino acids and other nitrogenous compounds including ammonium-L-glutamate were added to the basal diet (at the expense of the glucose component) and also were offered ad libitum to groups of six or more weanling rats.

The results are shown in Table 65. As can be seen from the Table, ammonium-L-glutamate was one of the six sources of "non-essential" nitrogen which, when individually added to the basal diet, provided the best growth, about 3g per day.

3. In 1958 Wilson and Koeppe (8033) showed that when glutamate was

Table 65 (8324)

Average Growth Response of Six Male, Sprague-Dawley Weanling Rats per Group when Individual Sources of "Non-Essential" Nitrogen were Added to the Basal Diet [Table I of Ref. (7)]

Diet No.	Component added	Total N of component added	Time on diet	Average starting weight	Average weight gain over period	Average intake over period		Ratio of weight gain to intake
		g.	days	g.	g.	ml.	g.	
39	None	0	21	50	9.0	192	96	0.09
1	L-Alanine	15.7	21	47	60.5	326	163	0.37
38	L-Proline	15.7	21	50	46.7	270	135	0.35
8	L-Arginine.HCl	15.7	24	46	58.5	382	191	0.31
9	D-Arginine.HCl	15.7	20	46	27.3	254	127	0.21
36	Glycine	15.7	21	49	14.5	166	83	0.17
37	L-Hydroxyproline	15.7	21	49	−2.4	108	54	—
31	L-Butyrine	15.7	21	49	7.5	130	65	0.12
32	D-Alanine	15.7	21	49	40.2	280	140	0.28
33	L-Serine	15.7	21	48	−8.0	80	40	—
7	L-Cysteine	15.7	—	45	All died	—	—	—
29	Urea	15.7	21	49	24.8	266	133	0.18
30	Ammonium acetate	15.7	21	49	48.2	314	157	0.32
10	L-Alanine	12.5	21	45	61.2	318	159	0.38
12	Ammonium L-glutamate	12.5	21	45	62.2	330	165	0.37
13	L-Glutamine	12.5	21	45	61.3	336	168	0.37
14	Ammonium L-aspartate	12.5	21	45	62.2	342	171	0.37
15	L-Asparagine.H_2O*	12.5	21	45	(56.0)	(360)	(180)	(0.31)

* Slow precipitation of L-asparagine from the diet solution rendered results doubtful.

injected i.p. into rats, conversion to succinate via α-ketoglutarate was the primary route of catabolism (see Fig. 7). When glutamic acid was intubated, part of it was converted to succinate via α-ketoglutarate, and part of it was metabolized to acetate via a pathway in which carbon 2 became the methyl carbon of acetate. When glutamic acid was instilled into the cecum, carbon 2 again became the methyl carbon of acetate. This conversion was considered to be due to the action of intestinal bacteria.

3. In 1971 Prosky and O'Dell (5932) studied the effects of dietary MSG on some brain and liver metabolites. Fifty male Holtzman albino weanling rats were fed cereal diets supplemented with MSG at 0, 1, 5, 10, and 20% levels (w/w) for 16 weeks. Brains and livers were then analyzed for glutamate-related metabolites.

Some effects of the feeding of MSG on the rat liver were:

(1) Aspartate concentrations were elevated compared with controls.

(2) Lactate and malate levels were not significantly changed.

(3) No significant changes were noted in liver protein, RNA-P, or DNA-P.

(4) An upward trend in the concentrations of liver glutamate and α-GPO_4 was observed.

Some effects of the feeding of MSG on the rat brain were:

(1) An observed hyperirritability in all rats receiving MSG.

(2) A significant decrease in GABA levels along with a significant increase in succinate levels.

(3) Levels of glutamate, aspartate, glutamine, DNA, protein, and glutamic acid decarboxylase remained relatively constant. Rats fed the 20% MSG-supplemented diet did not grow as well as control rats or those on the lower MSG supplementations.

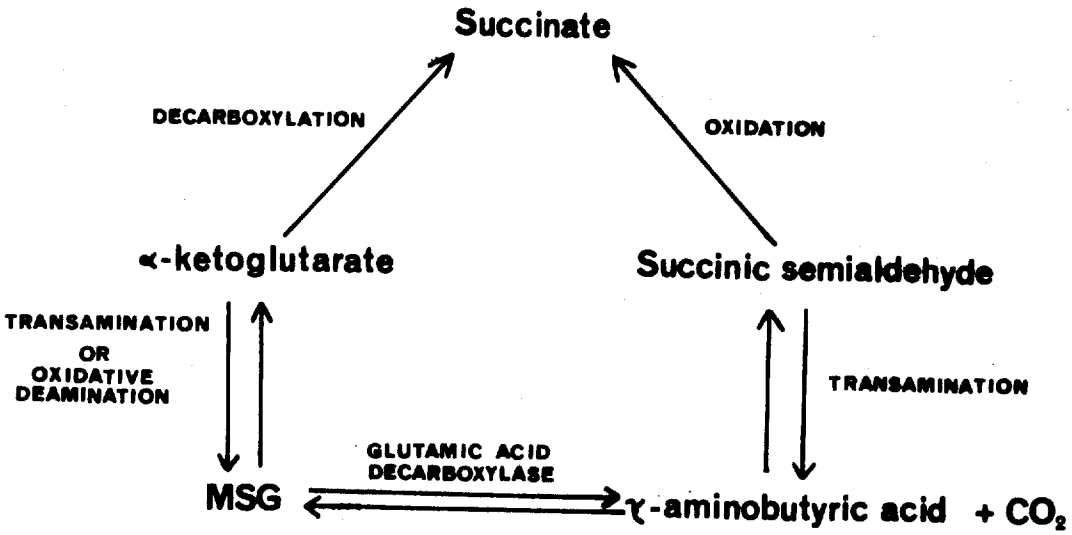


Fig. 7. The metabolic pathway of MSG in the rat brain. (8033)

4. In 1955 Gayer and Klingmuller (2411) studied the in vivo effect of glutamic acid on carbohydrate metabolism by the behavior of the liver glycogen in rats after the administration of 10 mmoles/kg MSG in 10% solution i.p. to 10 male albino rats (150-200 g) which were:

(1) Normal animals, or

(2) Animals whose adrenal function was blocked by a 0.5 mg/kg s.c. injection of Hydergine (an adrenolytic preparation of hydrogenated ergot alkaloids), or

(3) Adrenalectomized animals receiving 0.4 ml/100 g adrenal extract intramuscularly twice daily for 8 days after bilateral adrenalectomy and an additional dose of 10 mmoles/kg MSG, or

(4) Animals with pyridoxine deficiency.

They observed that:

(1) A significant reduction of liver glycogen occurred in normal rats by two hours after the last of two or three injections of 10 mmoles/kg MSG. The same MSG dose given in a single injection caused no significant reduction.

(2) Previous s.c. injection of Hydergine did not suppress the mobilization of glycogen after MSG administration. There was a reduction of blood sugar to levels judged hypoglycemic (55-85 mg/100 ml) in the experimental animals. The controls treated with glutamate or Hydergine alone showed no blood sugar alteration.

(3) Mobilization of glycogen and hypoglycemic convulsions occurred in the adrenalectomized rats at various times after two i.p. injections of 10 mmoles/kg MSG.

(4) Liver glycogen was not significantly mobilized in the pyridoxine-

deficient animals after MSG administration.

The authors concluded that the mobilization of liver glycogen by MSG was independent of any adrenaline effect, but an intact transaminase system was required.

5. In 1972 Prosky and O'Dell (8348) investigated the effects of dietary MSG on some selected brain and liver metabolites of second generation neonatal rats born to parents fed a diet supplemented with 10% MSG. Holtzman weanling rats of both sexes (number not stated) were fed laboratory chow either alone or supplemented with 10% MSG for 100 days, then mated. The offspring of these rats were continued on the same diet for 100 days and then mated. Ten second generation rats were then sacrificed at days, 1, 2, 3, 5, 10, and 21. The following determinations were carried out: (1) GAD, GABA, glutamate, aspartate, protein, and DNA in the brain; (2) RNA, DNA, protein and glutamate in the liver; and (3) the stomach contents of the 5-day-old rats were assayed for glutamate as an indication of MSG content of the mother's milk.

The results of the determinations in the brain showed only that GABA was significantly elevated on day 1, but by day 2 the values were normal. There was no effect observed in liver protein, RNA-P, DNA-P or glutamate. The stomach contents of 5-day old rats showed the presence of 20% more free glutamate in the stomachs of offspring of parents fed the MSG diet than in the stomachs of control offspring. This was thought to be the cause of the brain GABA elevations. Treated offspring had rough, shaggy coats which persisted for about 30 days.

The authors considered these results to be consistent with their earlier findings (5932, see p. 170). In generation F4, the only observed effect was the rough, shaggy coat described above.

6. In 1972 Prosky and O'Dell (8349) also studied the effects of dietary MSG on the activity of three brain enzymes in the F4 generation rats. The procedures were the same as before (8348) until the birth of the F4 generation. Then six to eight neonates from each group were sacrificed on days 1, 3, 5, 10, and 21. The effects on body weights as well as brain weights, protein contents and enzyme activities are summarized in Table 66. The authors noted that body and brain weights of treated rats were significantly higher than those of controls at various times during the first 21 days of neonatal development.

The effects of dietary MSG on brain levels of GOT, GDH, and GPT are summarized in Fig. 8. During the neonatal period it was observed that GOT and GPT activity increased 20-fold while GDH activity increased about 15-fold. The authors pointed out that, as can be seen in Table 66, during days 1 through 21 the glutamate-metabolizing enzymes were being synthesized at 2-3 times the rate of total protein synthesis in the brain. This study was carried out on the whole brain.

7. A decrease in serum cholesterol and associated β-lipoproteins was reported in 1969 by Bazzano (0580) when Mongolian gerbils (40-80 g) and 3-week-old chickens were fed an amino acid formula diet containing the eight essential (for humans) amino acids plus, for some groups, glutamic acid as the sole source of nonessential nitrogen. The gerbils showed the greater changes. (See Tables 67, 68 and 69). Adult rabbits (1.5-2.5 kg BW) fed the same diets did not respond, and adult male rats (St. Louis University Colony Doisy-Wistar strain 200-300 g BW) showed paradoxically higher serum cholesterol levels when fed the high glutamate diet. (See Tables.) The same author also reported hypolipemic effects in humans (0581, and see pages 114 and 192).

Table 66. Effect of Dietary MSG on Body Weights, Brain Weights and Protein Content, and Activities of GOT, GPT and GDH in Brains of Fourth-Generation Rats (8349)

Group	Body weight (g)	Brain weight (g)	Protein/brain (mg/g)	GOT activity (units)/protein mg	GPT activity (units)/mg protein	GDH activity (units)/mg protein
			1-day-old			
Control	6·48 ± 0·15	0·2631 ± 0·0038	78·7 ± 1·2	141 ± 6	6·2 ± 0·4	2·16 ± 0·35
Treated	5·98 ± 0·20	0·2712 ± 0·0048	75·8 ± 1·1	147 ± 4	6·6 ± 0·8	3·28 ± 0·77
			3-days-old			
Control	7·61 ± 0·16	0·3549 ± 0·0039	75·2 ± 0·8	153 ± 7	8·1 ± 1·7	1·62 ± 0·38
Treated	9·41 ± 0·20*	0·3972 ± 0·0101*	73·0 ± 0·6	183 ± 4*	7·4 ± 0·4	1·64 ± 0·38
			5-days-old			
Control	11·47 ± 0·28	0·5129 ± 0·0073	74·8 ± 1·4	187 ± 8	8·2 ± 0·6	3·40 ± 0·36
Treated	12·54 ± 0·40*	0·5681 ± 0·0148*	74·6 ± 1·0	178 ± 7	8·3 ± 0·6	3·27 ± 0·49
			10-days-old			
Control	20·87 ± 0·54	0·9191 ± 0·0116	83·9 ± 1·6	248 ± 9	10·6 ± 0·8	3·15 ± 0·40
Treated	22·25 ± 1·38	0·9539 ± 0·0289	82·3 ± 0·8	252 ± 8	10·3 ± 0·4	2·40 ± 0·26
			21-days-old			
Control	49·20 ± 1·71	1·4027 ± 0·0270	107 ± 2	458 ± 14	14·1 ± 0·8	4·97 ± 0·54
Treated	55·09 ± 2·64	1·4980 ± 0·0278*	110 ± 1	360 ± 16*	15·2 ± 0·6	4·64 ± 0·56

Each value represents the mean ± S.E.M. of analyses on 6–8 brains.
Enzyme activity is expressed in units, as defined in text section on methods.
* Mean differs significantly from that of control group, $P < 0.01$.

Table 67 (0580)

Serum Cholesterol Values ± S.E.M. in Animals Maintained for 2 Weeks on the Designated Diet

Animal	Dietary regimen		
	FCD[a]	AAF	AAFG
Chick	145 ± 6.7 (21)	167 ± 10.7 (12)	124 ± 6.9 (12)[b]
Rabbit	59 ± 10.5 (5)	----	79 ± 10.1 (5)
Rat	77 ± 7.2 (4)	81 ± 3.9 (12)	102 ± 3.7 (10)[c]
Gerbil	128 ± 11.2 (8)	138 ± 14.6 (12)	80 ± 4.2 (12)[d]

[a]Commercial rations (FCD), after addition of lard to bring the crude fat content up to 10%, contained: (a) for gerbils and rats: crude protein > 23.0%, crude fiber > 6.0%; (b) for rabbits and chicks: crude protein > 15%, crude fiber > 18%.

[b]AAFG serum cholesterol significantly ($P < 0.02$) lower than AAF.

[c]AAFG serum cholesterol significantly ($P = 0.005$) higher than AAF.

[d]AAFG serum cholesterol significantly ($P = 0.002$) lower than AAF; all other differences were insignificant at $P = 0.05$.

Number of animals in each experiment given in parentheses.

Table 68 (0580)

Serum Cholesterol Values ± S.E.M. in Gerbils Fed the Designated Diets, for 7-Day Periods

	Dietary regimen[a]					
	FCD	AAF	AAFG	AAF	AAFG	FCD
Period no.	1	2	3	4	5	6
Mean serum cholesterol (mg/100 ml)	155 ± 12.9 (14)	169 ± 11.1 (14)	80 ± 8.4 (14)	115 ± 11.2 (14)	63 ± 6.2 (10)	101 ± 9.6 (4)
Significance of change		$P = 0.35$	$P < 0.0005$	$P < 0.01$	$P < 0.005$	$P < 0.01$

[a]Number of animals in each experiment given in parentheses.

Table 69 (0580)

Effects of Various Diets on Mature Male Mongolian Gerbils[a]

Diet	Animals (No.)	Weight (g)	Δ-Cholesterol (mg/100 ml)	Observations
FCD	110	72 ± 1.0		Hair is smooth; occasional convulsions during handling
Casein (30%)	49	72 ± 1.1	- 8 ± 7.8	Hair loss; increased number of convulsions; increased mortality rate
AAF control	56	60 ± 1.2		Hair ruffled with occasional hair loss; rare hyperactivity, ataxia without additional convulsions
AAFG experimental	56	65 ± 1.0	-53 ± 14.0	As above

[a]Observations on test diets were recorded at the end of 1 week. Weight is reported as the mean ± S.E.M. Food control diet (FCD) was Purina laboratory chow for small animals; 20 of the gerbils received FCD with 1% (by weight) of cholesterol added. See text for casein diet; 26 of the gerbils on this diet received 30% casein with 1% (by weight) of cholesterol added. (Δ-Cholesterol represents the difference in concentration of cholesterol from that in animals on the preceding diet ± S.E. of the difference.) Amino acid formula (AAF) was fed to 56 gerbils, 28 of which received AAF with 1% (by weight) of cholesterol added. For animals fed amino acid formula with glutamate (AAFG), the statistical evaluation is limited to the AAF-AAFG interaction ($P < 0.005$). (Δ-Cholesterol represents the difference in concentration of cholesterol from that in animals on the preceding diet ± S.E. of the difference.) Twenty-eight of these gerbils received AAFG with 1% (by weight) of cholesterol added.

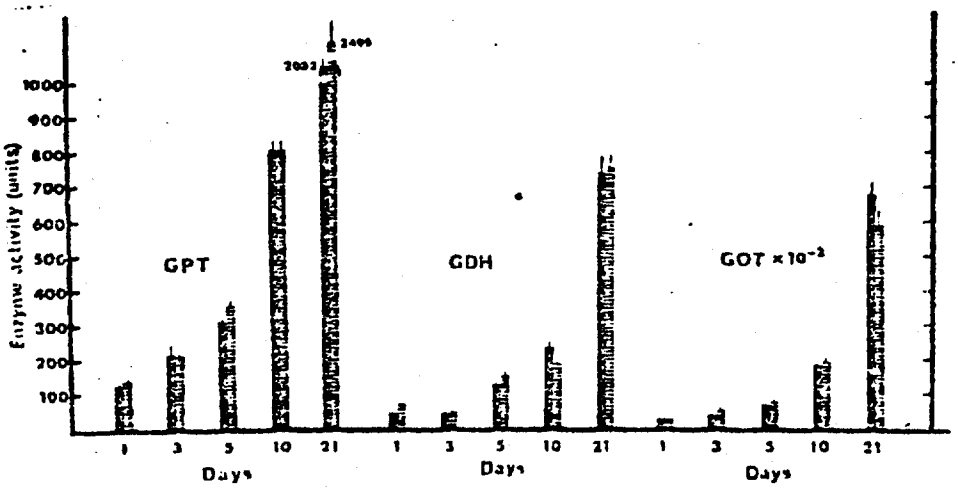


Fig. 8. Activities of GOT, GPT and GDH in whole brain homogenate of control (solid bars) and treated (lined bars) rats. These values were determined from initial reaction rats over a 10-min period when the change in optical density, measured at 5-s intervals with a Gilford 2000 multiple sample spectrophotometer is linear. Vertical lines = S.E.M. **(8349)**

C. Rabbits

1. In 1955 Klingmuller and Vogelsang (3829) studied the influence of L(+)-glutamic acid on the citric acid cycle by parallel determinations of the α-ketoglutaric acid and glutamic acid levels in the blood. MSG 300 mg/kg was given i.v., and MSG 500 mg/kg was administered i.p. to healthy rabbits (3-3.5 kg). The α-ketoglutaric acid concentration in the serum increased from its normal value of 0.195-0.325 mg% to 1.90-3.18 mg% after i.v. injection and to 4.30-4.75 mg% after i.p. administration. This indicated that the concentration of α-ketoglutaric acid in the serum was dose-dependent. The increase of α-ketoglutaric acid was accompanied by a simultaneous decrease of blood pyruvic acid corresponding to 88-96% of the α-ketoglutaric acid increase. The free ammonia content of the blood did not increase.

When a quantity of ammonium chloride (100 mg/kg) equimolar to the injected glutamate was administered i.v. there was no decrease in the serum concentration of pyruvic acid, even with a 13 mg% increase of serum free ammonia. The authors interpreted this as implying transamination (which they demonstrated in vitro could occur in the blood as well as in the liver) rather than oxidative deamination of glutamate followed by secondary amination of pyruvate.

2. Klingmuller et al. (3831) in 1955 reported further studies on glutamic acid metabolism by determining the fate of the amino nitrogen since no free ammonia could be detected in the blood in the first experiments (3829), in which serum α-ketoglutaric acid levels rose while pyruvic acid levels fell correspondingly after administration of MSG. Fully grown rabbits were given 2 mmoles/kg MSG both orally and i.v. These doses were equivalent to those used in the first experiment and, theoretically, also to a therapeutic dose of 20 g MSG given orally or i.v. to a 70 kg human (3831).

There was an increase of serum L-alanine, about 5-10 times the decrease in pyruvic acid. In addition it was found that:

(1) Doubling the dose of injected MSG did not further increase the L-alanine levels.

(2) Simultaneous administration of pyruvate with the MSG had no further effect on alanine concentrations.

(3) Oral MSG increased the L-alanine levels comparably with MSG i.v., after a delay due to absorption.

The authors concluded that the pyruvic acid substrate for the increase of serum alanine probably was derived from carbohydrate metabolism, and that there was a limited capacity for transamination which was completely exhausted by the 2 mmoles/kg of MSG administered.

3. Gayer and Klingmuller (2411), in a third paper of 1955, reported some in vivo effects of glutamate on the blood glucose curves of rabbits treated with:

(1) Glucose,

(2) Insulin,

(3) Alloxan.

The observations were:

(1) Simultaneous oral administration of 2 mmoles/kg L-glutamic acid with 4 or 8 mmoles glucose/kg depressed the blood glucose increase.

(2) When L-glutamic acid 2 mmoles/kg was given orally about 30 minutes before insulin 3 I.U. s.c., hypoglycemic shock was prevented.

(3) L-glutamic acid 2 mmoles/kg i.v. or 4 mmoles/kg orally in the alloxan group caused 70-200 mg% increases of blood glucose. The higher the initial blood glucose concentration, the greater the increase. The

increases were prevented by previous s.c. injections of 0.5 mg/kg Hydergine.

4. Petersen et al. (5745) in 1955 confirmed many of the findings reported by Klingmuller and co-workers (3829,3831,2411) in rabbits administered 2 g/kg MSG.

(1) MSG given s.c. (route preferred over stomach tube) had a marked hyperglycemic effect. The authors concluded that an increase of blood glutamate concentration in normal animals disturbed the homeostatic control of blood sugar level. There was a decrease of liver glycogen but no change in blood pressure.

(2) The MSG dose protected hypoglycemic rabbits (injected intramuscularly with 8-12 I.U. insulin) from death.

(3) The blood concentration of α-ketoglutaric acid increased while that of pyruvic acid decreased.

D. Goats

In 1970 Egan et al. (1961) studied the metabolic fate of ^{14}C-labeled L-glutamic acid injected i.v. as a pulse dose in lactating goats (one Saanan and two Toggenbergs). Glutamic acid was rapidly and extensively oxidized. Respired CO_2 contained about 40% ^{14}C from the glutamate in the first three hours. More of the ^{14}C appeared in lactose than in milk protein. The authors concluded that in the goat, processes involving gluconeogenesis acquired a greater part of the glutamate label than processes involved in milk protein synthesis.

E. Cows

Egan et al. (1960) reported in 1968 on the metabolic fate of i.v. injected ^{14}C-labeled glutamic acid in the lactating cow. They found that

during milk formation the amount of glutamate carbon utilized for carbohydrate synthesis was greater than that utilized for protein synthesis. They proposed as an explanation that the rate and extent of glutamate catabolism in the cow was greater than the rate and extent of its utilization for protein synthesis.

F. Pigs

1. In 1972 Baker et al. (8322) studied the effect of MSG loading on the newborn pig. This is an abstract with very little experimental detail given.

Labeled MSG dissolved in water or in infant formula was administered to 3-day old pigs by stomach tube, at dose levels of 0.01 and 0.1 g/kg BW. Tissue samples (from what part of the animal was not stated), peripheral blood, and portal blood were obtained with time for amino acid analyses. The observations reported were that significant quantities of radioactivity in the amino acid fraction were found only in glutamate, glutamine, and alanine. The plasma and tissue levels of these amino acids were not found to be significantly different from those of control animals. The authors concluded that "it seems unlikely that any neurotoxic effect would be observed at the dose levels studied".

2. In 1973 Stegink et al. (8365) studied MSG metabolism in 3-day-old pigs fasted five to six hours (for absorption studies see 8364, p. 149). Glutamate-U-C^{14} was given in water or infant formula at 1 g/kg by tube.

Label was found rapidly in glutamate, glutamine, arginine, aspartate, alanine, ornithine, citrulline, urea, and also in glucose and lactate, with 65-80% in glutamate, glucose, and lactate, of which the glutamate was the most rapidly removed from the plasma. Traces were found in plasma pyruvate and α-ketoglutarate, but not in succinate, malate, citrate, oaxaloacetate, or pyrrolidone carboxylate. Neither labeled glutamate nor aspartate entered the

spinal fluid as such, although label was found in spinal glutamine, glucose, lactate, and urea. For typical data see Figure 9 and Table 70.

On the reliability of analyses the authors noted that use of label allowed measurement of the flux of particular atoms without identifying the pertinent compounds; most amino acids were converted in vivo to ninhydrin-negative compounds that could not be measured by analyzer techniques, although they could be separated by the analyzer and then measured by the label. For the authors' techniques see the paper itself.

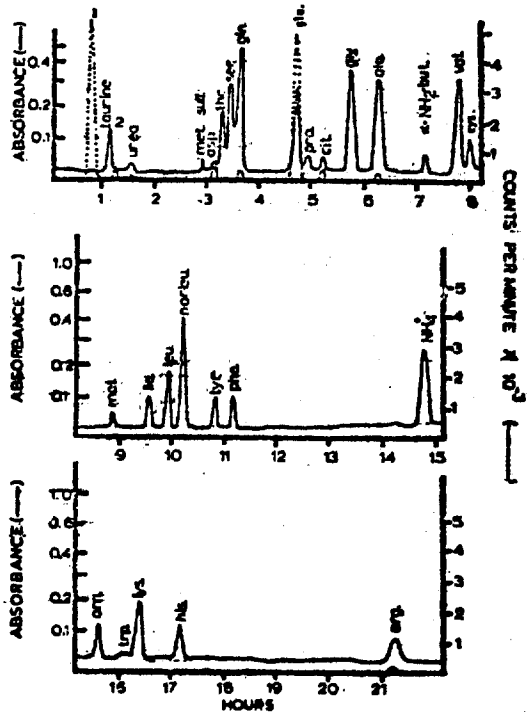
The authors proposed pathways for glutamate metabolism as shown in Fig. 10. They found that glutamate that entered the liver went to the mitochondria where it entered the TCA cycle; the label was transferred via oxaloacetate to aspartate which was returned to the blood. Labeled malate went to PEP, and most of the PEP went to glucose, but some went via pyruvate to lactate. Some of the α-ketoglutarate went to CO_2, and some of the CO_2 went via carbamyl-phosphate to urea. Alanine was probably formed from glutamate in the blood. The authors suggested that since peptides were absorbed from the gut more rapidly than were free amino acids, the form in the gut might determine the balance of eventual metabolites. They considered it unlikely that glutamate itself would be returned by the liver to the blood.

The authors commented on the data of Creasey and Malawista (1547, see p. 79), that their conclusion of decreased brain uptake of glucose following glutamate pretreatment could be explained by simple dilution of the labeled glucose pool with glucose derived from unlabeled glutamate.

G. Monkeys

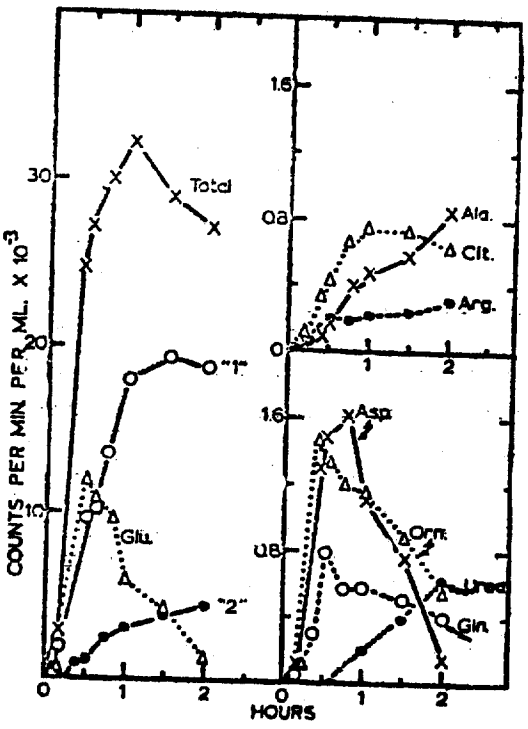
1. In 1967 Kerr and Waisman (8343) investigated the transplacental ratios for serum amino acid in the rhesus monkey during pregnancy. The amino acid

Fig. 9



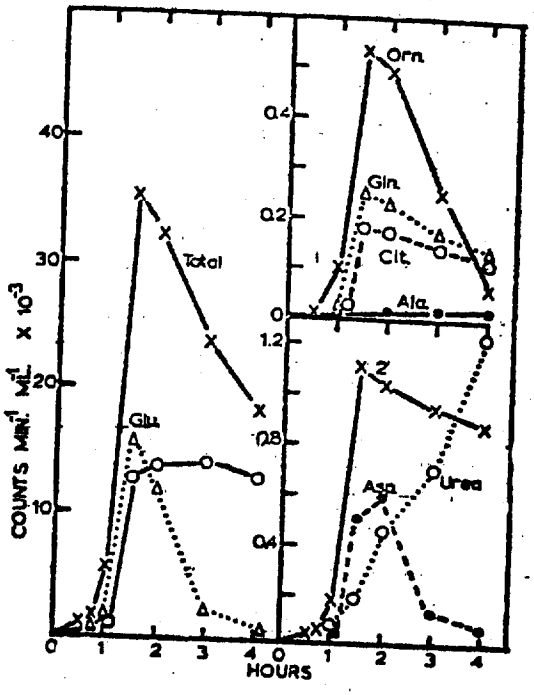
A. A typical simultaneous radioactivity-amino acid analysis elution profile of plasma obtained 60 minutes after administration of MSG in water using the specially modified amino acid analyzer described (13). The abscissa lists the elution time from the analyzer column in hours. The ordinate lists both the absorbance of the eluate at 570 nm following reaction with ninhydrin (solid line) and the radioactivity detected (dotted line). The minor radioactivity peaks are drawn larger than actual size in order to demonstrate the definite presence of label at those positions. The precise radioactivity data are shown in B and C. **(8365)**

Fig. 9 (cont.)



B. Mean labeling rate of plasma metabolites following administration of a 1 g/kg load of MSG and 10 μCi of U-^{14}C-MSG in water to three neonatal pigs. Variability about each point does not exceed 15%. **(8365)**

Fig. 9 (cont.)



C. Mean labeling rate of plasma metabolites following administration of a 1 g/kg load of MSG and 10 μCi of U-^{14}C-MSG in infant formula to three neonatal pigs. Variability about each point does not exceed 15%. **(8365)**

Table 70

Radioactivity Profile in Physiological Fluids of a Neonatal Pig Loaded with 10 µCi ^{14}C-Monosodium Glutamate (8365)

Compound	Plasma	Spinal fluid
	cpm/ml	
Aspartate	640	ND*
Glutamine	220	130
Glutamate	13,573	30
Alanine	100	30
Ornithine	550	ND
Arginine	33	ND
Citrulline	180	ND
Glucose	14,200	11,460
Lactate	990	630
Urea	420	360

*ND = not detected.

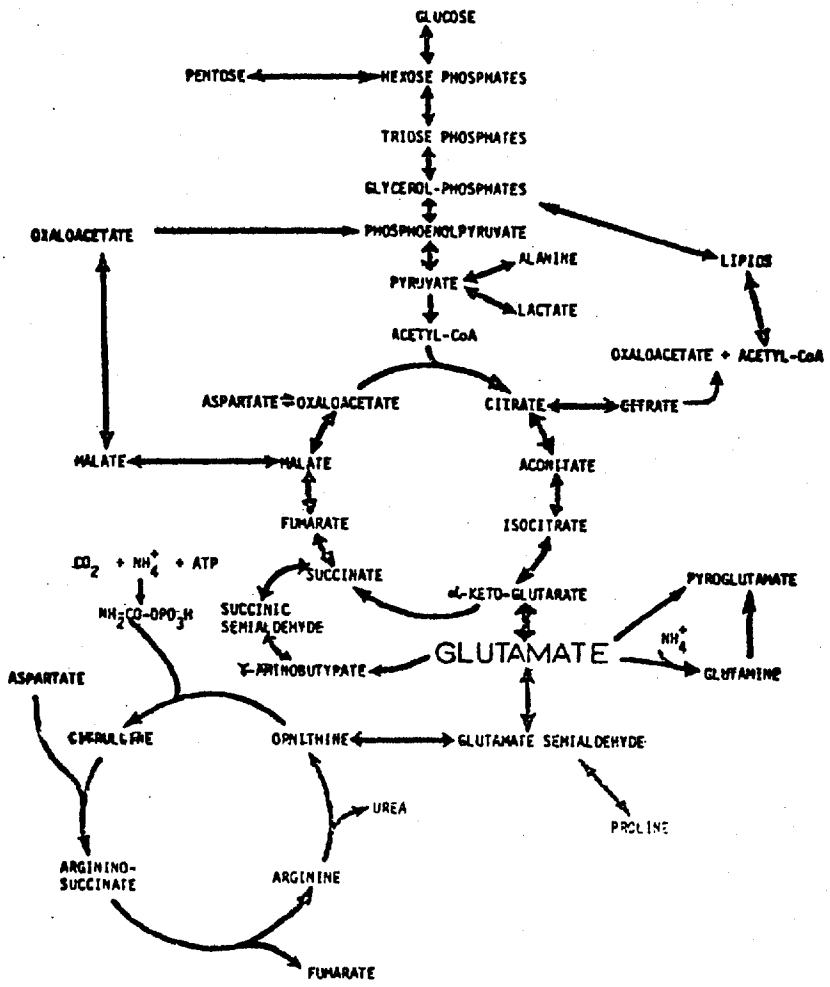


Fig. 10. Available pathways of glutamate metabolism (8365)

blood levels of fetuses and mothers were studied during pregnancy at six gestational ages. Then at delivery, simultaneous blood samples were drawn from the umbilical vein of the infant and the maternal vena cava. The serum amino acid concentration of the pregnant rhesus monkeys at term was also compared with that of non-pregnant rhesus monkeys.

The authors found that the pregnant animals had a lowered serum concentration of most amino acids, including glutamic acid, aspartic acid, and glutamine. The ratio of fetal to maternal serum levels of amino acids was found to be higher earlier in gestation for most of the common amino acids and to approach unity as term was reached. The primate placenta functioned to maintain a higher blood level of amino acids in the fetus than in the mother. Though suited to periods of maternal deprivation, this mechanism would ensure that excessive maternal levels were magnified in the fetus. Infants so born had been found nutritionally and neurologically normal, yet preliminary tests had revealed behavioral defects that were being studied (8343).

2. In 1971 Pitkin et al. (8350) investigated whether labeled glutamic acid crossed the primate placenta. This is an abstract in which very little experimental detail is given. L-glutamic acid-3,4-^{14}C was administered to pregnant rhesus monkeys. Serial maternal and fetal plasma samples were then analyzed on an amino acid analyzer.

The observations reported were that 68% of the radioactivity in the maternal plasma remained associated with glutamate while 22% was converted to a pre-taurine ninhydrin-negative acidic compound, and small amounts were converted to a post-taurine ninhydrin-negative acid, aspartate, glutamine, and ornithine. The two acidic compounds were found to contain 80% of the radio-

activity in the fetal plasma and less than 2% of the counts were associated with glutamate.

From these observations, the authors concluded that the hemochorial placenta was virtually impermeable to L-glutamic acid and that at sometime during the process of placental transfer or earlier in the maternal system, glutamate was metabolized to acidic compounds, not identified, which then traversed the placenta.

Further work from this laboratory on the absorption and metabolism of MSG was reported in 1972 in humans (8354, see p. 160) and in 1973 in pigs (8365,8364, see pp. 149 - 184).

H. Humans

1. When Olson et al. (5500) fed four human subjects formula diets of L-amino acids in the proportions and amounts found in mixed diets (16.0 g N) instead of protein, they observed no significant changes in serum cholesterol or β-lipoproteins from control values. If, however, at the same N intake, the mixture fed was altered to include the eight essential amino acids, plus glutamate as the sole source of nonessential nitrogen, then:

(1) Serum cholesterol fell 31 mg per 100 ml (30-60 mg%).

(2) Phosphatides fell 19 mg per 100 ml.

(3) β-Lipoproteins fell 73 mg per 100 ml.

(4) Triglycerides increased 49 mg per 100 ml.

(5) All subjects remained in nitrogen balance throughout the experiment.

The authors concluded that the hypolipidemic effect was not due to nitrogen imbalance but rather that the formation of β-lipoproteins by the liver was a function of the nonessential as well as the essential amino acid nitrogen in the diet.

2. The hypocholesterolemic effect of glutamic acid in humans fed a chemically defined formula diet was studied further in the same laboratory by Garlich et al. (2395). They observed that when equimolar amounts of glycine or ammonium acetate (or citrate) replaced the dietary glutamate there was no hypocholesterolemic effect. An additional observation was that in subjects receiving 137 g of glutamate daily, there was an elevation of alanine but not glutamate in their plasma (see also 3831, pp. 180-181).

3. To study the mechanism of the observed hypocholesterolemic effect of glutamate, Olson et al. (5499) labeled the cholesterol pool by administering either a labeled precursor of cholesterol or labeled cholesterol itself. They then observed the change in slope of the biological decay curves in patients fed the glutamate-containing diet (AAFG) described in the first report from this laboratory (5500) or the diet in which glycine and ammonium acetate replaced the glutamate (AAF) described in the second report (2395).

The results showed that with the AAFG diet there was a decrease in the ratio of fecal sterols to bile acids. The authors concluded from the data obtained on cholesterol turnover during the switch from the AAF to the AAFG diet, as well as altered sterol balance, that the fall in serum cholesterol and its associated low density lipoproteins was caused by a decrease in the entry of cholesterol molecules into the bile plasma pool. They noted, however, that the precise biochemical mechanism was unclear and further study was needed.

4. In a 1970 study in which Bazzano et al. (0581) fed 25-147 g of MSG per day to 14 adult human subjects (see p. 114), the average fall observed in plasma cholesterol was 42±15.8 (SEM) mg/100 ml (see Table 35). The authors concluded that glutamate metabolically decreased the serum cholesterol, phospholipids, and β-lipoproteins.

IV. Effects on Enzymes and Other Biochemical Parameters

The *in vivo* effects of the glutamates on cellular and tissue metabolism and morphology have been described in the Biological Sections II-V and the Biochemistry Sections I-III.

V. Drug Interaction

A. Mice

An additive effect has been reported for MSG combined with sodium L-aspartate and implied for MSG plus cysteine and MSG plus glutamic acid.

1. In 1970, Olney and Ho (5492) found that Webster Swiss albino mice given single oral doses of 0.50/kg MSG plus 0.5 g/kg sodium L-aspartate developed similar degrees of dose-dependent brain lesions as did others given 1.0 g/kg of either compound alone. This study is reported on pp. 69-71.

2. In 1972 Olney (5482) (see p. 122) reported a synergistic effect between MSG and three other acidic amino acids. When 10-day-old mice (number, sex, and strain not stated) were given s.c. 0.75 mmole/kg separately of MSG, or the sodium salt of L-aspartic, cysteic or cysteine sulfinic acids, no changes were seen in the arcuate nucleus. When a combined dose of these four agents, each at a dose of 0.75 mmole/kg (total dose of acidic amino acids = 3.0 mmoles/kg) was given, a small lesion was produced in the arcuate nucleus that was judged equivalent to a lesion that would have been produced by any of these four substances given separately at 3.0 mmoles/kg. When a combination of these four agents each at a dose of 3 mmoles/kg (total dose of acidic amino acids = 12 mmoles/kg) was given, a large lesion involving essentially the entire arcuate nucleus was produced. This was judged equivalent to a lesion which would have been produced by any of these substances given separately at 12 mmoles/kg (5482).

B. Rats

1. In 1971 Weiss et al. (7915) in a study of motor activity concluded that MSG at 10% in the diet diminished the responses of rats to amphetamine.

2. In 1970 Knittle and Ginsberg-Fellner (3859) found that MSG altered the responses of rat adipose tissue to epinephrine and to insulin (see pp. 82-83).

C. Guinea Pigs and Rabbits

A potentiating effect was observed in 1955 by Klosa (3835) who found that the B complex vitamins and vitamin B_6 in particular appeared to enhance the efficacy of glutamic acid and MSG (to a lesser extent) at preventing cramps induced by isonicotinic acid hydrazide.

Known toxic doses of isonicotinic acid hydrazide were given to five guinea pigs (600 mg/kg toxic dose) and 5 rabbits (700-800 mg/kg toxic dose). Table 71 shows the doses of various substances which, when given orally after administration of the "lethal" dose of isonicotinic acid hydrazide, prevented death or cramps to a greater or lesser degree in the test animals (3835 in translation).

D. Humans

1. Levey et al. (4346) (see p. 118) administered i.v. (1) five different amino acid preparations of known glutamic acid content (three protein hydrolysates, two amino acid mixtures) to 47 male subjects, (2) solutions containing only partly neutralized glutamic acid to 31 male subjects. They found that fewer of the second group developed nausea and vomiting than of the first group receiving the mixtures with free glutamic acid. This observation was interpreted by the authors as showing that the toxicity of glutamic acid could be potentiated by other amino acids (see pp. 118, 122 for more details).

Table 71 (3835)

No.	Dose (mg/kg)	Substance
I	600	L-Glutamic acid (salt-free)
II	580	Monosodium glutamate
III	720	Disodium glutamate
IV	720	L-Glutamic acid hydrochloride
V	430	L-Glutamic acid with 5 μg B_{12}
VI	420	L-Glutamic acid with 5 μg B_{12}, 100 μg folic acid
VII	480	L-Glutamic acid with 300 μg B_1
VIII	340	L-Glutamic acid with 800 μg B_1, 5 μg B_{12}, 600 μg B_6, and 100 μg folic acid
IX	410	Bioglutan (2) (Contains the entire vitamin B complex and vitamin B_{12})
X	400	Monosodium glutamate and 400 μg B_6

2. In 1972 Upton and Barrows (7596) reported a case of CRS in an epileptic in which they suspected that the MSG effect had been potentiated by diphenylhydantoin therapy (see p. 116).

3. Effects of glutamic acid on the circulatory system led to a caution against its indiscriminate use in i.v. injection formulations (4761, see p. 140).

VI. Consumer Exposure Information

Monosodium glutamate has been used in the Orient for several centuries, since it occurs naturally in a powdered seaweed used as a flavoring, and in soy sauce. However, it did not become commercially available on any scale in the United States until the early 1950's. Since then, enthusiastic promotion by manufacturers has made the presence of monosodium glutamate in prepared foods almost ubiquitous. Its use by restaurants is not limited to those specializing in Chinese cooking, for the National Restaurant Association in 1955 endorsed and encouraged the use of MSG in the public feeding industry (4459). The success of this effort is seen in increased production figures. Over 75,000 tons were reported produced by firms surveyed by the National Academy of Sciences in 1971 -- a figure that represents 60-70% of the actual poundage added to the nation's food supply according to NAS (5225). Table 72 lists poundages reported for glutamic acid, glutamic acid hydrochloride, MSG, MAG, and MKG. MAG and MKG are used primarily as components of salt substitutes.

The effectiveness of MSG depends on the salt content of the food and its inherent level of glutamate (2802). MSG is most effective in a food that has some salt content, and is added to Oriental foods in proportion to the amount of salt added, as Table 73 shows (0251). This can vary from 10 to 30% of the salt content of the food (0251). In the presence of such thickening agents as gums, flour, and starches, higher glutamate levels are required than in

Table 72 (5225)

Substance	1960	1970	Total 1970 lbs. Report to NAS	FEMA	Total 1970 lbs. NAS and FEMA
Glutamic acid	33	4,110	4,508	1,276	5,784
Glutamic acid Hcl	40,000	40,000	40,000	----	40,000
Monoammonium glutamate	400	4,000	4,000	15,000	19,000
Monopotassium glutamate	0	40,000	85,316	666	85,982
Monosodium glutamate	23,901,400	143,978,521	146,047,498	4,010,987	150,058,485 (75,029 tons)

Table 73. Standard Amounts To Be Used for Japanese Dishes (0251)

Name of dish	Monosodium glutamate %	Salt (%)
Clam soup flavored with salt	0.10	1.0
Chawan-mushi (thick custardy soup)	0.12	1.0
Sumashijiru (clear soup, Japanese soup)	0.10 - 0.15	1.0
Soup for tempura	0.50	3.5
Soup for Sukiyaki	0.7 - 1.5	7.0
Deep-boiled taro	0.24	1.2
Vegetables boiled hard with soy sauce	0.45	1.8
Fried eggs	0.20	1.0
Pork boiled with vinegar	0.45	1.5
Kanitama (crab flesh fried with egg and vegetables)	0.20	1.0
Frizzled boiled rice mixed with pork and vegetables	0.24	0.8
Chow mein (frizzle noodles) mixed with pork and vegetables	0.30	1.0
Soup with balls of pounded shrimps	0.12	1.0
Cold noodles	0.20	1.0
Pork and vegetable boiled in thick soy sauce with sugar	0.30	1.5
Shao-mai	0.20	0.8

unthickened products (2802). A level of 0.15% is considered proper for most western foods but others may require as much as 0.24% (2802). The flavor enhancing property of MSG is also diluted by fat (2802). The mechanism of this property is unknown (1476).

Publicity surrounding the Chinese Restaurant Syndrome made the general public aware of the heavy use of MSG by Oriental restaurants, but in fact its use as a flavor enhancer is more widespread. Foods to which MSG is not added before sale to consumers include liquid milk, some unprocessed cheeses, fresh fruits, some candies, and some baked goods. MSG is added to frozen and canned vegetables, fresh cuts of beef, pork, and veal, ground hamburger and pork, frozen or canned pork and chicken products, fresh and prepared seafood, canned meat products such as gravies and stews, dehydrated and canned soups, salad dressings, roasted nuts, canned or frozen foods containing cheese, and popcorn and potato chips (1476).

Table 74 lists some typical usages for glutamate products, and their percentages. While manufacturers of canned and frozen foods list MSG on their product labels, no such information appears on fresh meat, such as poultry which has been dusted with MSG shortly after slaughter (2538).

The NAS Food Safety Committee which was given the responsibility of evaluating the merits of MSG in baby foods based its recommendation for removal on the fact that the MSG conferred no direct benefit to the infant, adding that the levels of MSG in a typical food item were no higher than 0.6% (5226). However, Olney et al. (5485) pointed out that at this level one small jar of baby food (130 g) would provide about 0.78 g of MSG or 0.13 g/kg of body weight for a human infant weighing 6 kg. Since Olney et al. found that an oral dose of 1 g/kg in the primate (0.5 g/kg in the mouse) was sufficient to

Table 74. Usage Levels[a] (2538)

Substance		Usual use wtd. mean, %	Max use wtd. mean, %
Monoammonium glutamate	Soups	16.61684	17.42716
Glutamic acid	Baked goods(R)	.01353	.01682
Glutamic acid	Frozen dairy(R)	.00080	.00160
Glutamic acid	Meat products(R)	.00281	.00967
Glutamic acid	Condiment relish(R)	.06803	.10869
Glutamic acid	Soft candy(R)	.00160	.00320
Glutamic acid	Gelatin pudding(R)	.00040	.00080
Glutamic acid	Beverage type I(R)	.00020	.00040
Glutamic acid	Beverage type II(R)	.00040	.00080
Glutamic acid	Seasoning flavors(R)	.26664	.27019
Monopotassium glutamate	Seasoning flavors(R)	1.07465	1.07934
Monopotassium glutamate	Misc. unclass.(R)	.20000	.47000
Monosodium glutamate	Baked goods(R)	.18861	.28420
Monosodium glutamate	Breakfast cereals(R)	.26000	1.07000
Monosodium glutamate	Other grains(R)	.29384	.49360
Monosodium glutamate	Fats oils(R)	.05638	.10495
Monosodium glutamate	Milk products(R)	.05024	.10029
Monosodium glutamate	Cheese(R)	.10015	.15015
Monosodium glutamate	Processed fruit(R)	.15313	.15313
Monosodium glutamate	Meat products(R)	.08513	.19984
Monosodium glutamate	Poultry(R)	.08410	.22942
Monosodium glutamate	Egg products(R)	.19700	.19700
Monosodium glutamate	Fish products(R)	.08335	.16974
Monosodium glutamate	Processed vegetables(R)	.23725	.45148
Monosodium glutamate	Condiment relish(R)	.24755	.50220
Monosodium glutamate	Soft candy(R)	.00013	.00030
Monosodium glutamate	Soups(R)	.41818	.80343
Monosodium glutamate	Snack foods(R)	.07002	.40447
Monosodium glutamate	Beverage type I(R)	.00414	.00414
Monosodium glutamate	Nut products(R)	.10375	.20407
Monosodium glutamate	Reconstituted vegetables(R)	.19954	.40656
Monosodium glutamate	Gravies	.38951	.73398
Monosodium glutamate	Seasoning flavors (R)	23.38409	24.67852
Monosodium glutamate	Misc. unclass.(R)	.17363	.28552

[a] (R) indicates regular foods.

destroy hypothalamic neurons, this left a 4- to 8-fold margin of safety for a human infant eating one jar, a 2- to 4-fold margin if two jars were eaten, etc. -- less than one order of magnitude (5485).

According to a Food and Agriculture Organization/World Health Organization (FAO/WHO) report (3440), infants aged 5-6 months and weighing 7.5 kg consume 500 g of cow's milk and 2 jars of baby food a day, giving them a daily intake of bound glutamic acid of 3.5 g in the former and 0.5 g in the latter. The corresponding free glutamic acid intake is 0.015 and 0.060 g/day, equivalent to 0.62 g/kg body weight of glutamic acid per day. If two jars of baby food (200 g) contain 0.3% MSG, the total intake of free glutamic acid is increased from 0.06 to 0.60 g. In a survey of children aged 9-12 months the intake of baby food over seven days was found to range from 0 (20% of cases surveyed) to a maximum of 250 g daily (Berry, unpublished reports, Dept. of Health and Social Security, London, quoted in 3441).

In 1970 the FAO/WHO Expert Committee on Food Additives reported on MSG (3441). They concluded: "In view of the uncertainty regarding the possible susceptibility of the very early human neonate to high oral intakes of glutamate, it would be prudent not to add monosodium glutamate to foods specifically intended for infants under one year of age."

In 1971 the same Committee extended their caution to additives in general in foods that might be eaten by human infants (3440). They pointed out that non-milk formulas formed "an appreciable proportion of the calorie intakes" at 3-6 months, after which the infant was "increasingly fed from the family meals". Very young children tended to lack the metabolic protective mechanisms present in adults, and also the blood brain and blood retinal barriers, so that "there may be deleterious consequences that may not appear until much

later in the child's development" (3440).

The Committee (3440) specifically recommended:

(1) Basic research into methods for testing additives for infant foods, with special attention to pre-weaning differences between humans and test animals.

(2) Study of any additives essential to baby foods.

(3) Continual reassessment of the safety of such additives.

(4) Investigation of criteria for justifying additives used in baby foods.

(5) In such safety evaluations "the possibility should be borne in mind that certain classes of foods containing additives may be consumed by infants as well as by adults."

Those recommendations can be compared with the exposures estimated in the NAS GRAS survey (Table 75).* The possible average daily intake for infants in the 0-5 month group is 6.4 mg of MSG in baked goods, 1.5 mg in other grain, and 2.7 mg in milk products intended for adult consumption (keyed with an R in the Tables). Possible total daily intakes estimated by the NAS are for infants 0-5 months, 33.2 mg MAG and 25.7 mg MSG: 6-11 months, 3871.7 mg MKG and 2290.8 mg MSG 12-23 months, 5782.7 mg MAG, 714.1 mg MSG, and 10.5 mg glutamic acid.

The FAO/WHO Expert Committee pointed out that while sodium and chloride were necessary nutrients in infant formulas, if sodium chloride were added to other classes of baby foods for taste purposes it should be limited to 0.24%

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* These tables are lengthy computer printout sheets and are not reproduced in full in this monograph.

Table 75. Possible Daily Intakes of NAS Appendix A Substances (Groups I and II), per Food Category and Total Dietary, Based on Food Consumption by Total Sample[a]

Substance name (survey no.)	Food category no. name	# of firms	(Age)	Possible daily intake, mg Average	High A	High B
Glutamic acid NAS 0087	01 Baked goods (R)	-	0-5 mo.	.460020	.608850	.571880
			6-11 mo.	3.436620	7.008540	4.272280
			12-23 mo.	7.373850	12.149940	9.166900
			2-65+ yr.	18.563160	27.574140	23.077040
Glutamic acid NAS 0087	07 Frozn dairy (R)	-	0-5 mo.	.008000	.032800	.016000
			6-11 mo.	.076000	.211200	.152000
			12-23 mo.	.115200	.270400	.230400
			2-65+ yr.	.204800	.493800	.409600
Glutamic acid NAS 0087	10 Meat prods (R)	-	0-5 mo.	.030910	.081490	.106370
			6-11 mo.	.581670	1.567980	2.001690
			12-23 mo.	.848620	1.458390	2.920340
			2-65+ yr.	2.203040	3.655810	7.581280
Glutamic acid NAS 0087	15 Condm relsh (R)	-	0-5 mo.	---	.068030	---
			6-11 mo.	.544240	1.496660	.869520
			12-23 mo.	1.904840	5.170280	3.043320
			2-65+ yr.	5.986640	14.422360	9.564720
Glutamic acid NAS 0087	16 Soft candy (R)	-	0-5 mo.	.003200	.032000	.006400
			6-11 mo.	.035200	.108800	.070400
			12-23 mo.	.056000	.148800	.112000
			2-65+ yr.	.092800	.281600	.185600
Glutamic acid NAS 0087	20 Gelatin pud (R)	-	0-5 mo.	.008000	.010800	.016000
			6-11 mo.	.051200	.155200	.102400
			12-23 mo.	.055200	.134400	.110400
			2-65+ yr.	.081600	.210000	.163200

Table 75 (Cont'd)

Substance name (survey no.)	Food category no. name	# of firms	(Age)	Possible daily intake, mg Average	High A	High B
Glutamic acid NAS 0087	23 Bev type I (R)	-	0-5 mo.	.004800	.007200	.009600
			6-11 mo.	.045400	.155400	.090800
			12-23 mo.	.108400	.325000	.216800
			2-65+ yr.	.208000	.555400	.416000
Glutamic acid NAS 0087	24 Bev type II (R)	-	0-5 mo.	.000000	.000000	.000000
			6-11 mo.	---	.000400	---
			12-23 mo.	---	.000800	---
			2-65+ yr.	.130000	.377600	.260000
Glutamic acid NAS 0087	48 Seas flavrs (R)	-	0-5 mo.	---	---	---
			6-11 mo.	---	.026664	---
			12-23 mo.	---	.053328	---
			2-65+ yr.	.026664	.133320	.027019
Glutamic acid NAS 0087	All categories	8	0-5 mo.	.514930	.841170	.726250
			6-11 mo.	4.770330	10.730844	7.559090
			12-23 mo.	10.462110	19.711338	15.800160
			2-65+ yr.	27.496704	47.703830	41.684459
Monoammonium glutamate NAS 0129	21 Soups (R)	-	0-5 mo.	33.233680	249.252600	34.854320
			6-11 mo.	3871.723720	12080.442680	4060.528280
			12-23 mo.	5782.660320	15968.783240	6064.651680
			2-65+ yr.	5267.538280	14041.229800	5524.409720
Monoammonium glutamate NAS 0129	All categories	-	0-5 mo.	33.233680	249.252600	34.854320
			6-11 mo.	3871.723720	12080.442680	4080.528280
			12-23 mo.	5782.660320	15968.783240	6064.651680
			2-65+ yr.	5267.538280	14041.229800	5524.409720
Monopotassium glutamate NAS 0133	48 Seas flavrs (R)	-	0-5 mo.	---	---	---
			6-11 mo.	---	.107465	---
			12-23 mo.	---	.214980	---
			2-65+ yr.	.107465	.537325	.107934

Table 75 (Cont'd)

Substance name (survey no.)	Food category no. name	# of firms	(Age)	Possible daily intake, mg Average	High A	High B
Monopotassium glutamate NAS 0133	All categories	-	0-5 mo.	---	---	---
			6-11 mo.	---	.107665	---
			12-23 mo.	---	.214930	---
			2-65+ yr.	.107465	.537325	.107934
Monosodium glutamate NAS 0134	01 Baked goods (R)	18	0-5 mo.	6.412740	8.487450	9.662800
			6-11 mo.	47.906940	97.699980	72.186800
			12-23 mo.	102.792450	169.371780	154.889000
			2-65+ yr.	258.772900	384.387180	389.922400
Monosodium glutamate NAS 0134	03 Other grain (R)	14	0-5 mo.	1.469200	4.995230	2.468000
			6-11 mo.	28.502480	84.038240	47.879200
			12-23 mo.	48.189760	111.365360	80.950400
			2-65+ yr.	81.687520	180.417760	137.220300
Monosodium glutamate NAS 0134	04 Fats oils (R)	9	0-5 mo.	.281900	.281900	.524750
			6-11 mo.	1.578640	4.228500	2.938600
			12-23 mo.	3.551940	6.765600	6.611850
			2-65+ yr.	9.866500	17.816080	18.366250
Monosodium glutamate NAS 0134	05 Milk prods (R)	-	0-5 mo.	2.712960	2.009600	5.415660
			6-11 mo.	31.349760	150.770240	62.580960
			12-23 mo.	27.380800	87.618560	54.658050
			2-65+ yr.	19.844800	60.589440	39.614550
Monosodium glutamate NAS 0134	06 Cheese (R)	4	0-5 mo.	---	.100150	---
			6-11 mo.	2.704050	9.714550	4.054050
			12-23 mo.	7.811700	22.233300	11.711700
			2-65+ yr.	9.414100	23.635400	14.114100
Monosodium glutamate NAS 0134	08 Procsd frut (R)	-	0-5 mo.	7.197110	19.294380	7.197110
			6-11 mo.	79.321340	197.537700	79.321340
			12-23 mo.	154.048780	305.800610	154.048780
			2-65+ yr.	181.152790	383.743780	181.152790

Table 75 (Cont'd)

Substance name (survey no.)	Food category no. name	# of firms	(Age)	Possible daily intake, mg Average	High A	High B
Monosodium glutamate NAS 0134	10 Meat prods (R)	62	0-5 mo.	.936430	2.468770	2.198240
			6-11 mo.	17.621910	47.502540	41.366880
			12-23 mo.	25.709260	44.182470	60.351680
			2-65+ yr.	66.741920	110.754130	156.674560
Monosodium glutamate NAS 0134	11 Poultry (R)	21	0-5 mo.	.420500	1.934300	1.147100
			6-11 mo.	3.279900	11.101200	8.947380
			12-23 mo.	5.550600	15.474400	15.141720
			2-65+ yr.	10.848900	27.504800	29.595180
Monosodium glutamate NAS 0134	12 Egg prods (R)	-	0-5 mo.	---	1.970000	---
			6-11 mo.	.985000	3.546000	.985000
			12-23 mo.	1.773000	7.683000	1.778000
			2-65+ yr.	3.743000	13.593000	3.743000
Monosodium glutamate NAS 0145	13 Fish prods (R)	12	0-5 mo.	.083350	.250050	.169740
			6-11 mo.	1.083550	4.084150	2.206620
			12-23 mo.	4.500900	11.252250	9.165960
			2-65+ yr.	10.335400	25.755150	21.047760
Monosodium glutamate NAS 0134	14 Procsd vegs (R)	17	0-5 mo.	3.321500	9.964500	6.320720
			6-11 mo.	56.940000	132.860000	108.355200
			12-23 mo.	92.527500	154.924250	176.077200
			2-65+ yr.	201.662500	339.742000	383.758000
Monosodium glutamate NAS 0134	15 Condm relsh (R)	11	0-5 mo.	---	.247550	---
			6-11 mo.	1.980400	5.446100	4.017600
			12-23 mo.	6.931400	18.813800	14.061600
			2-65+ yr.	21.784400	52.480600	44.193600
Monosodium glutamate NAS 0134	16 Soft candy (R)	-	0-5 mo.	.000260	.002600	.000600
			6-11 mo.	.002860	.008840	.006600
			12-23 mo.	.004550	.012090	.010500
			2-65+ yr.	.007540	.022880	.017400

Table 75 (Cont'd)

Substance name (survey no.)	Food category no. name	# of firms	(Age)	Possible daily intake, mg Average	High A	High B
Monosodium glutamate NAS 0134	21 Soups (R)	26	0-5 mo.	.836360	6.272799	1.606860
			6-11 mo.	97.435940	304.016860	187.199190
			12-23 mo.	145.526640	401.870980	279.593640
			2-65+ yr.	132.563060	353.362100	254.687310
Monosodium glutamate NAS 0134	22 Snack foods (R)	10	0-5 mo.	---	.070020	---
			6-11 mo.	.280080	.770220	1.617880
			12-23 mo.	.770220	2.170620	4.449170
			2-65+ yr.	.910260	2.590740	5.258110
Monosodium glutamate NAS 0134	23 Bev type I (R)	-	0-5 mo.	.099360	.149040	.099360
			6-11 mo.	.939780	3.216780	.939780
			12-23 mo.	2.243880	6.727500	2.243880
			2-65+ yr.	4.205600	11.496780	4.305600
Monosodium glutamate NAS 0134	25 Nut prods (R)	7	0-5 mo.	---	.207500	---
			6-11 mo.	3.838750	13.902500	7.550590
			12-23 mo.	2.801250	9.337500	5.509890
			2-65+ yr.	5.395000	16.081250	10.611640
Monosodium glutamate NAS 0134	26 Reconst veg (R)	4	0-5 mo.	---	---	---
			6-11 mo.	---	---	---
			12-23 mo.	---	---	---
			2-65+ yr.	.399080	1.197240	.813120
Monosodium glutamate NAS 0134	27 Gravies (R)	28	0-5 mo.	.389510	1.168530	.733980
			6-11 mo.	5.453140	15.190890	10.275720
			12-23 mo.	14.022360	39.730020	26.423280
			2-65+ yr.	32.329330	82.96563	60.920340
Monosodium glutamate NAS 0134	02 Break cerls (R)	-	0-5 mo.	1.960000	4.420000	6.420000
			6-11 mo.	57.980000	155.480000	238.610000
			12-23 mo.	67.860000	132.340000	279.270000
			2-65+ yr.	52.000000	134.680000	214.000000

Table 75 (Cont'd)

Substance name (survey no.)	Food category no. name	# of firms	(Age)	Possible daily intake, mg Average	High A	High B
Monosodium glutamate NAS 0134	48 Seas flavrs (R)	10	0-5 mo.	---	---	---
			6-11 mo.	---	2.338409	---
			12-23 mo.	---	4.676818	---
			2-65+ yr.	2.338409	11.692045	2.467852
Monosodium glutamate NAS 0134	All categories	112	0-5 mo.	25.721180	64.294320	43.964920
			6-11 mo.	439.184520	1243.453699	881.039390
			12-23 mo.	713.996990	1552.350908	1336.941300
			2-65+ yr.	1106.103029	2234.587985	1972.484362

[a] (R) indicates regular foods.

by weight of the product (3440).

While MSG has been withdrawn from baby foods by manufacturers, there are no current limits on its use in foods intended for adults. Tables 75 and 76 (5225) present possible average and high daily intakes of glutamates for five age categories in the foods surveyed. Glutamic acid and MKG are used relatively lightly; MAG occurs mostly in soups. The greatest average intake of MSG is derived from baked goods, processed vegetables and fruits, and soups. MSG is an important ingredient of clear soups. One manufacturer lists in a recipe for consomme, 17 g salt, 3 g sugar, 9.38 g lactose and 4 g MSG to 0.1 g beef extract, 0.6 g of carrot, and 1 g of onion (0251).

A sector of the consuming public that has been particularly affected by the discovery of MSG's properties consists of the men and women in the armed services. Service personnel first encountered MSG in the form of soy sauces flavoring the rations of Japanese soldiers in World War II (3281). With the encouragement of the glutamate industry in the United States, the Quartermaster Food and Container Institute tested the acceptability of MSG (4490). Favorable responses led the Army, Navy, Marine Corps, and Air Force to include MSG in their recipe publications as an ingredient to enhance flavoring. However, published reports of adverse effects of MSG, characterized as the Chinese Restaurant Syndrome (CRS), led in 1968 to a reexamination of MSG by the Armed Forces Product Evaluation Committee. Upon the recommendation of the Surgeon General, the use of MSG in Armed Forces recipes was reduced to a minimum. A typical 30-day Armed Forces menu guide suggests using 4 oz of MSG per 100 men or 1.24 g/man/30 days, or 40 mg/day/man. The Armed Forces Recipe Service, which is utilized by all four services, now lists MSG as an optional

Table 76. Potential Daily Intakes of NAS Appendix A Substances (Groups I and II) per Food Category Based on Food Consumption by Eaters Only (Numbers rounded off)

Food category	Substance name	Age	Potential daily intake, mg Average	High A	High B	Very High
Other grain than breakfast cereals	Monosodium glutamate	0-5 mo.	26.1	54.7	44.0	91.9
		6-11 mo.	53.1	122.0	89.3	204.9
		12-23 mo.	58.4	116.7	98.2	196.0
		2-65+ yr.	100.4	197.7	168.8	332.1
Baked goods	Glutamic acid	0-5 mo.	2.0	7.0	2.7	8.7
		6-11 mo.	3.9	7.3	4.9	9.1
		12-23 mo.	7.4	12.1	9.2	15.1
		2-65+ yr.	18.5	27.5	23.0	34.2
Breakfast cereals	Monosodium glutamate	0-5 mo.	13.2	34.5	54.5	142.3
		6-11 mo.	86.0	169.2	354.1	696.5
		12-23 mo.	73.9	133.7	303.9	550.0
		2-65+ yr.	69.7	144.3	386.8	593.9
Processed vegetables	Monosodium glutamate	0-5 mo.	19.5	37.2	37.0	70.9
		6-11 mo.	71.6	139.3	136.3	265.0
		12-23 mo.	95.8	155.9	182.4	296.6
		2-65+ yr.	202.8	340.2	306.0	647.4
Condiment relish	Glutamic acid	0-5 mo.	.27	.408	.434	.652
		6-11 mo.	1.2	2.3	1.9	3.7
		12-23 mo.	2.9	6.6	4.6	10.5
		2-65+ yr.	7.4	15.6	11.8	24.9
Condiment relish	Monosodium glutamate	0-5 mo.	.9	1.5	2.0	3.0
		6-11 mo.	4.5	8.4	9.0	17.0
		12-23 mo.	10.3	24.0	21.0	48.7
		2-65+ yr.	26.9	56.7	54.7	115.0

Table 76 (Cont'd)

Food category	Substance name	Age	Potential daily intake, mg Average	High A	High B	Very High
Soups	Monoammonium glutamate	0-5 mo.	697.9	1163.2	731.9	1219.9
		6-11 mo.	8025.9	18610.9	8417.3	19518.4
		12-23 mo.	8241.9	17929.6	8643.9	18803.9
		2-65+ yr.	8408.2	17730.2	8818.1	18594.8
Fats, oils (R)	Monosodium glutamate	0-5 mo.	2.8	7.8	5.1	14.6
		6-11 mo.	2.3	4.7	4.3	8.7
		12-23 mo.	3.7	6.8	6.8	12.7
		2-65+ yr.	10.0	17.9	18.7	33.3
Milk products (R)	Monosodium glutamate	0-5 mo.	26.2	56.9	52.3	113.6
		6-11 mo.	66.0	200.8	131.7	400.8
		12-23 mo.	42.4	154.5	84.6	308.4
		2-65+ yr.	28.5	78.0	57.0	155.9
Cheese	Monosodium glutamate	0-5 mo.	.7	1.2	1.0	1.8
		6-11 mo.	8.7	16.3	13.0	24.5
		12-23 mo.	11.5	24.9	17.2	37.4
		2-65+ yr.	13.0	27.1	19.5	40.7
Processed fruit	Monosodium glutamate	0-5 mo.	38.7	99.2	38.7	99.2
		6-11 mo.	102.6	226.9	102.6	226.9
		12-23 mo.	179.8	326.5	179.8	326.5
		2-65+ yr.	197.2	393.4	197.2	393.4
Poultry	Monosodium glutamate	0-5 mo.	8.4	21.0	22.9	57.4
		6-11 mo.	9.1	17.3	25.0	47.3
		12-23 mo.	10.7	18.3	29.4	50.0
		2-65+ yr.	17.1	33.0	46.8	89.9
Egg products	Monosodium glutamate	0-5 mo.	4.7	6.5	4.7	6.5
		6-11 mo.	8.7	14.6	8.7	14.6
		12-23 mo.	11.2	23.4	11.2	23.4
		2-65+ yr.	17.1	31.3	17.1	31.3

Table 76 (Cont'd)

Food category	Substance name	Age	Potential daily intake, mg Average	High A	High B	Very High
Fish products	Monosodium glutamate	0-5 mo.	2.7	4.9	5.4	10.0
		6-11 mo.	3.4	6.4	6.9	13.0
		12-23 mo.	7.3	13.0	14.9	26.6
		2-65+ yr.	15.8	29.9	32.0	60.9
Nut products	Monosodium glutamate	0-5 mo.	.4	.5	.8	1.0
		6-11 mo.	11.5	24.3	22.5	48.0
		12-23 mo.	6.0	14.0	11.8	27.5
		2-65+ yr.	11.3	24.5	22.2	48.3
Reconstituted vegetables	Monosodium glutamate	2-65+ yr.	3.6	6.0	7.3	12.1
Gravies	Monosodium glutamate	0-5 mo.	4.3	8.1	8.0	15.4
		6-11 mo.	12.0	23.1	22.8	43.3
		12-23 mo.	27.3	54.5	51.3	102.8
		2-65+ yr.	51.8	102.9	97.6	193.8
Seasoning flavorings	Monosodium glutamate	0-5 mo.	--	--	--	--
		6-11 mo.	4.7	9.3	4.9	9.9
		12-23 mo.	7.0	11.7	7.4	12.3
		2-65+ yr.	23.4	46.8	24.7	49.4

ingredient in its recipes.*

There are as many suggested usage levels for MSG as there are manufacturers and products, although it is recognized in the food industry that there is a point at which further addition of MSG does not enhance flavor. Levels recommended by the Food, Drugs, and Cosmetic Act of 1940 range from 0.05% to about 1.5%, with most levels at 0.15-0.25%. The highest levels occur in soups and soup mixes and are about 0.14-0.128 g per serving (2182).

An FDA estimate based on 1967 tariff schedule figures for production of MSG places the consumption of MSG at about 0.23 lb (104 g) per person per year. Possible average daily intakes for glutamates estimated from the NAS/NRC GRAS survey (5225) are: MAG 5267.5 mg; MSG 1106.1 mg; and glutamic acid 27.5 mg. However, intakes of 1500 mg MSG have been found to trigger allergic reactions in sensitive individuals (3676).

Thus, many experts have recommended that consumer exposures be restricted until safe levels have been defined by specific experimental evidence (3436, 5485,1091,4761).

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* Private communications from J. Niland, Armed Forces Recipe Service.

PROTEIN HYDROLYSATES

CHEMICAL INFORMATION

I. Nomenclature

A. Common names: Hydrolyzed protein, protein hydrolysates, hydrolyzed plant protein (HPP), hydrolyzed vegetable protein (HVP)

B. Chemical names: None

C. Trade names: Vegamine, Kerateme, Luxor, Protex, Griffith, Maggi, Vico Products-Dry, Staley-Liquid, Travamin, Aminosol, C.P.H.

II. Empirical Formula

Because of the nature of hydrolyzed protein, it is not possible to derive an empirical formula. Instead, shown in Tables 77 (5689) and 78 (5689) are typical analytical compositions.

III. Structural Formula

Hydrolyzed plant and animal proteins are composed mainly of a mixture of amino acids, the composition of the mixture depending on the source of the protein. Table 79 (5689) shows a typical amino acid composition for hydrolyzed plant protein.

IV. Molecular Weight

Not applicable to hydrolyzed protein.

V. Specifications

No specifications data available.

VI. Description

Hydrolyzed protein may exist as liquid, paste, or powder. It is available in colors ranging from light to dark. The powder form is hygroscopic and ideally should be handled under conditions where

Table 77. Typical Analytical Composition of Hydrolyzed Plant Protein (5689)

	Liquid	Paste	Powder
Total solids, %	40.0	85.0	98.0
Ash, %	20.0	42.5	49.0
Organic solids, %	20.0	42.5	49.0
Chloride, calc. as NaCl %	17.5	37.0	42.5
Total Nitrogen %	2.80	6.00	6.90
Protein (Nx6.25) %	17.5	37.5	43.0
MSG %	5.0	10.6	12.0
pH	5.3	5.2	5.1

Table 78. Typical Analytical Composition of Yeast Extract (5689)

	Standard Series (Dark & Light)		Special Series (Dark & Light)		Low Sodium Series	
	Paste	Powder	Paste	Powder	Paste	Powder
Total solids %	80.0	97.0	80.0	97.0	80.0	97.0
Ash %	23.0	28.0	22.0	26.5	10.0	12.0
Organic solids %	57.0	69.0	58.0	70.5	70.0	85.0
Chloride, calc. as NaCl %	15.0	18.0	13.0	15.7	2.0	3.4
Total nitrogen %	7.80	9.45	7.50	9.10	9.35	11.0
Protein (Nx6.25) %	48.7	59.5	46.9	57.0	58.5	69.0
MSG %	4.5	5.45	21.0	25.5	4.3	5.2
pH	5.5	5.5	5.5	5.5	5.6	5.6

Table 79. Amino Acid Composition of Hydrolyzed Plant Protein (5689)

	Brand X (%)	Brand Y (%)
Valine	1.7408	1.7485
Lysine	1.5624	0.8697
Threonine	1.4277	1.5002
Leucine	1.6003	2.0192
Isoleucine	1.0201	0.8037
Phenylalanine	1.2781	1.7268
Histidine	0.7007	0.8283
Arginine	1.4206	1.4030
Aspartic acid	3.0017	2.9841
Serine	1.8953	2.1891
Glutamic acid	20.2145	11.4599
Proline	3.0457	4.4480
Glycine	1.2542	1.1566
Alanine	3.2414	4.0773
Tyrosine	0.2965	0.2670
Total amino acid	43.700	37.421
Total protein (Nx6.25)	43.8	37.9

the relative humidity is less than 30%. The powder will usually fuse at temperatures above 95°F.

VII. Stability

Store well below 95°C and in moisture-protective packages. Mix only with ingredients having low moisture content to avoid caking. Combinations of protein hydrolysates with reducing sugars will often result in a Maillard browning reaction (5689).

BIOLOGICAL DATA

I. Acute Toxicity

See toxicity Table 12 in MSG section for lethal doses of casein hydrolysates.

Boyd et al. (0917) administered by intragastric cannula enzymatic casein hydrolysate dissolved in distilled water at body temperature to young male, albino rats (150-200 g) starved outright. Eleven doses increasing from 10 g/kg to 50 g/kg were each given to 16-20 rats. The interval to death (mean 3.6 $\pm$ 1.4 hours at LD_{50} dose of 26.0 $\pm$ 1.6 g/kg) was shorter the higher the dose. Death was due to respiratory failure in a cyanotic coma. Clinical signs of toxicity during the first hour were listlessness, cyanosis, and diarrheic bowel movements. On autopsy, an intense congestion of the brain, hemmorhagic inflammation of the gut, and a dark-colored liver were observed. Microscopic examination revealed vascular congestion in many organs and degenerative kidney and lung changes where delayed death occurred.

II. Short-Term Studies

A. Mice

Olney and Ho (5492) found that three of the amino acids present in protein hydrolysates (glutamate, aspartate, and cysteine) when given orally to mice (see Table 18, page 69) caused the development of both retinal and hypothalamic lesions in each animal treated with these compounds. Monosodium L-aspartate (MSA) and MSG were given by intubation (1 g/kg in 10% aqueous solution) to 4 Webster Swiss albino mice (10-12 days old) and 3 g/kg in 10% aqueous solution of L-cysteine was similarly administered to the same number of animals. When a mixture of MSG (0.5 g/kg) and MSA (0.5

g/kg) was orally administered to 8 animals, each developed hypothalamic damage to the same degree as seen in animals treated with 1 g/kg of either compound (see Table 21). This suggested to the authors that these two amino acids found in protein hydrolysates were additive in their toxic effect.

In an unpublished study reported in another paper, Olney et al. (5485) found that s.c. injection in 10-day-old mice of 0.2 ml protein hydrolysate produced a hypothalamic lesion unaccompanied by behavior disturbance.

Olney and Ho (5492) concluded that their experiments showed that at relatively low levels of oral intake, both glutamate and aspartate were toxic to the infant mouse, and when taken together these common amino acids had an additive brain-damaging effect. These researchers questioned the desirability of supplementing human infant diets with these substances.

Olney et al. (8347) compared the neurotoxicity of three commercial protein hydrolysates in mice. These were: Travamin, a casein enzymatic digest; CPH, a casein enzymatic digest; and Aminosol, an acid hydrolyzed fibrin. Groups of six mice were injected s.c. with undiluted hydrolysate solution at 20, 40, 60, 180, or 100 µl/g BW. At each dose level a control group was given a similar mixture of amino acids but without the acidic and sulphur amino acids shown to have brain-damaging properties. All the mice were sacrificed five hours after injection and the brain tissue processed as previously described by the authors (5483 and 5485).

The observations are summarized in Table 80. The authors did not observe any unusual behavior. They concluded that parenteral alimentation of human infants with casein hydrolysates did not afford a wide margin of safety against possible hypothalamic damage.

Table 80

Frequency* and Severity** of Hydrolysate-Induced Brain Damage (8347)

Solution	Dose (µl/g) 20	40	60	80	100
Control	0	0	0	0	0
Aminosol^{R}	0	0	0	2(+)	3(+)
CPH^{R}	0	4(+)	6(++)	6(+++)	6(+++++)
Travamin^{R}	1(+)	4(+)	6(++)	6(+++)	6(++++)

* The number of animals (out of six treated with each solution at each dose) sustaining hypothalamic damage.

** Necrotic neurons per representative section (NN/sec) per animal averaged for affected animals of each group. + = 1 to 10 NN/sec; ++ = 11 to 20 NN/sec; +++ = 21 to 30 NN/sec; ++++ = 31 to 40 NN/sec; +++++ = 41 to 50 NN/sec.

B. Rats

1. Boyd et al. (0917) found that hydrolyzed casein fed to rats produced toxic effects not produced by ingesting either casein itself or casein salts. The LD_{50} in rats of the enzymatic casein hydrolysate was approximately 26 g/kg as compared to 400-500 g/kg for the water-soluble sodium and calcium

salts of casein given over a 5-day period. Eleven groups of 16-20 overnight-starved young male albino rats (Wistar strain, 150-200 g) were fed by intragastric cannula enzymatic casein hydrolysate dissolved at body temperature in a volume of 100 ml/kg distilled water in doses of 10, 20, 22.5, 24, 25, 26, 27.5, 29, 30, 40, and 50 g/kg. Thirty-one controls received only distilled water. Casein hydrolysate, which is composed of amino acids (see Table 81) and polypeptides, is water-soluble and osmotically active. Oral doses of about 25 g/kg produced such toxic reactions in rats as violent gastroenteritis, withdrawal of water from tissues and blood, widespread capillary venous congestion, coma, and death within a few hours. (The observations at this toxic dose level are summarized in Tables 82, 83 and 84.)

Hydrolyzed casein is used in some proprietary infant formulas and in treating babies with special feeding problems, such as allergenic sensitivity to intact proteins or pancreatic deficiences (0917). Boyd et al. note that their experimental results suggest that if infants were fed casein hydrolysates in somewhat greater amounts than the recommended dosage (5 g/kg/day), toxic signs might be produced resembling the disease being treated.

2. Two toxicity studies were carried out by the Nestle Company (0249) on their hydrolyzed plant protein (HPP) designated 4BE. The analysis and amino acid content of this material are shown on Tables 85 and 86. In the first study, 20 male and 20 female SPF Sprague-Dawley rats were randomly divided into seven groups and fed diets containing high dosages of the HPP (see Table 87) for a period of 6 weeks. The results of this experiment showed that extremely high levels (50% in diet) caused severe

Table 81 (0917)

Free Amino Acid Composition of Protein Hydrolysate Preparations for Infusion

Amino acid	Amigen (casein) (micromoles/100 ml)
Taurine	Not detected
Cysteic acid	Not detected
Hydroxyproline	Not detected
Methionine sulfoxides	117
Aspartic acid	500
Threonine	922
Serine	1,900
Asparagine	Not detected
Glutamine	Not detected
Glutamic acid	1,960
Proline	1,280
Citrulline	5
Glycine	758
Alanine	1,109
Valine	1,686
1/2 Cystine	Not detected
Methionine	754
Isoleucine	1,365
Leucine	2,960
Tyrosine	234
Phenylalanine	1,182
Ornithine	40
Lysine	2,493
1-Methylhistidine	Not detected
Histidine	631
Arginine	165
Tryptophan	165

Table 82 (0917)

Changes in the Fresh Weight of Body Organs at Autopsy in Albino Rats Given Doses of Casein Enzymatic Hydrolysate in the Range of the Oral LD_{50}[a]

Organ	At death (N = 17 + 19 controls)	2-week survivors (N = 15 + 14 controls)	1-month survivors (N = 18 + 16 controls)
Adrenal glands	- 4.3	- 3.8	- 12.5[b]
Brain	- 3.2[c]	- 0.3	+ 0.1
Gastrointestinal tract:			
Cardiac stomach	- 24.1[b]	- 17.7[c]	+ 2.0
Pyloric stomach	- 21.2[b]	- 8.8	- 0.7
Small bowel	- 5.2	- 9.2	+ 9.5[b]
Cecum	- 19.7[b]	+ 4.9	- 0.1
Colon	- 18.9[b]	- 10.8[c]	+ 3.6
Heart	- 2.4	+ 2.9	- 3.4
Kidneys	- 5.2[c]	- 5.9[c]	+ 4.0
Liver	- 6.6	- 5.0	+ 8.4[c]
Lungs	+ 13.6	- 7.6[c]	+ 1.5
Muscle (ventral abd. wall)	- 30.8[b]	- 3.6	- 17.5[b]
Salivary glands (submax.)	- 2.3	+ 1.5	- 0.6
Skin	- 11.2[b]	- 0.5	- 2.9
Spleen	- 28.4[b]	- 8.5	- 7.8
Testes	- 17.1[b]	- 2.3	- 3.0
Thymus gland	- 17.4[c]	- 11.4[c]	- 7.9
Residual carcass	- 13.7[b]	- 3.7[c]	- 0.2
Total body wt	- 2.4	- 3.2[c]	- 0.7

[a]The organs were weighed in grams and the results are expressed as mean percent change from controls, specifically as $((\bar{X}_d - \bar{X}_c) \div \bar{X}_c) \times 100$ where $\bar{X}_d$ is the mean in the drug (casein)-treated rats and $\bar{X}_c$ in the respective controls.

[b]A mean difference significantly different from zero at $P = 0.01$ or less.

[c]A mean difference significantly different from zero at $P = 0.05$ to 0.02.

Table 83 (0917)

Changes in the Water Content of Body Organs at Autopsy on Albino Rats[a] Given Doses of Casein Enzymatic Hydrolysate in the Range of the Oral LD_{50}

Organ	At death (N = 17 + 19 controls)	2-week survivors (N = 15 + 14 controls)	1-month survivors (N = 18 + 16 controls)
Adrenal glands	- 24.6[b]	+ 8.3[c]	+ 7.6
Brain	- 15.2[b]	- 0.3	+ 0.2
Gastrointestinal tract:			
Cardiac stomach	- 41.0[b]	- 5.3	- 0.2
Pyloric stomach	- 41.9[b]	- 2.6	+ 3.2
Small bowel	- 30.3[b]	- 0.6	+ 0.8
Cecum	- 24.6[b]	+ 0.9	+ 2.5
Colon	- 33.2[b]	+ 1.0	+ 3.9[c]
Heart	- 17.3[b]	- 0.6	- 0.2
Kidneys	- 17.8[b]	- 0.7	- 0.4
Liver	- 17.0[b]	+ 0.5	- 6.0[c]
Lungs	- 16.0[b]	- 2.2	+ 1.2
Muscle (ventral abd. wall)	- 24.0[b]	+ 0.9	- 1.0
Salivary glands (submax.)	- 16.2[b]	+ 0.6	+ 2.5
Skin	- 23.6[b]	- 2.2	- 2.7
Spleen	- 11.3[b]	- 0.7	+ 0.7
Testes	- 17.6[b]	+ 1.6[c]	+ 0.6
Thymus gland	- 19.5[b]	- 2.2	+ 2.3
Residual carcass	- 21.3[b]	- 4.8[c]	+ 3.0

[a]Water content was measured as grams water/100 g dry weight of tissue and the results are expressed as mean percent change from controls, specifically as $((\bar{X}_d - \bar{X}_c) \div \bar{X}_c) \times 100$ where $\bar{X}_d$ is the mean in the drug (casein) treated rats and $\bar{X}_c$ in the respective controls.

[b]A mean difference significantly different from zero at $P = 0.01$.
[c]A mean difference significantly different from zero at $P = 0.05$ to 0.02.

Table 84 (0917)

Histopathologic Observations in Albino Rats at Death Due to Oral Administration of a Lethal Dose of Casein Enzymatic Hydrolysate

Organ	Histopathology
Adrenal glands	Sinusoidal erythrocytes packed and distorted; clotting, minute areas of early necrosis
Brain	Marked capillary-venous congestion and hemorrhages in the meninges and brain
Gastrointestinal tract:	
Cardiac stomach	Capillary-venous congestion of the submucosa with areas of lysis of the stratified squamous epithelium
Pyloric stomach	Capillary-venous congestion of the lamina propria and submucosa
Small bowel	Capillary-venous congestion of the lamina propria and submucosa and shrunken villi
Cecum	Capillary-venous congestion and hemorrhage of the lamina propria and submucosa and lysis of glands
Colon	Capillary-venous congestion of the lamina propria and submucosa
Heart	Coronary capillaries and veins congested and occasionally blood clots present
Kidneys	Vascular congestion, especially in the loop region and tubular fatty degeneration in late deaths
Liver	Sinusoids packed with distorted erythrocytes and areas of venous clotting

Table 84 (Cont'd)

Organ	Histopathology
Lungs	Venous clots in early deaths and areas of edema and hemorrhage in late deaths
Muscle	Fibers shrunken but otherwise normal in appearance
Salivary glands (submax.)	Normal appearance
Skin	Ischemic
Spleen	Red pulp shrunken, packed erythrocytes, venous clots
Testes	Tubules shrunken, extravascular clots, some tubular lysis
Thymus gland	Venous clots and some loss of thymocytes

Table 85 (0249)

Analysis of HPP 4BE Powder[a]

Total solids	97.5%	
Ash	51.7%	
Organic solids	45.8%	
Chloride, calc. as NaCl	39.6%	
Total nitrogen	4.74%	
Protein (N% x 6.25)	29.6%	
MSG	13.6%	
Ammonium chloride	1.25%	
pH	5.10%	
Bacteriological specification		
Standard plate count	7500	(/8 g)
Yeast and mold	50	(/ g)
Total thermophiles	150	(/10 g)
Flat	50	(/10 g)
Coliform	Negative	
Salmonella	Negative	

[a]Figures represent maximum limits.

Table 86 (0249)

Amino Acid Composition of Powdered Hydrolysate 4BE

Lysine	1.02%
Histidine	0.39%
NH_3	0.45%
Arginine	1.40%
Aspartic acid	2.32%
Threonine	1.02%
Serine	1.15%
Glutamic acid	11.90%
Proline	1.77%
Glycine	1.25%
Alanine	2.08%
Valine	1.38%
Methionine	0.24%
Isoleucine	0.71%
Leucine	1.17%
Tyrosine	0.27%
Phenylalanine	0.87%
Total amino acids	29.39%

Table 87 (0249)

Preliminary Trial[a]

Groups

A. Basal casein control (20% protein)

B. Basal casein diet + 10% Maggi powder 4BE

C. Basal casein diet + 20% Maggi powder 4BE

D. Basal casein diet + 50% Maggi powder 4BE

E. Basal casein diet containing 19.8% NaCl equal to D

G. Basal casein diet containing 0.65% NH_4Cl equal to D

H. Basal casein diet containing 6.8% Monosodium glutamate equal to D

[a]All diets contain 20% protein; appropriate adjustments were made in the amounts of casein in diets B, C and D, to take into account the protein contributed by Maggi powder 4BE.

clinical symptoms (severe diarrhea, weight loss, bad fur) and in some cases, death. The tested material was apparently more toxic at the 50% level than a quantity of sodium chloride alone equivalent to that contributed by 50% of the HPP 4BE.

The second experiment was carried out for 90 days. Twenty male and 20 female SPF Sprague-Dawley rats (85 g) were randomly divided into nine groups and fed the same basal diet as in the first experiment but with dosages of the HPP 4BE incorporated at lower levels of 1%, 2.5%, 5%, and 10% (see Table 88) corresponding to ingesting 1, 2.5, 5, and 10 g/kg of the protein hydrolysate.

Some observations were:

(1) The test diet tended to retard the growth of the males, with the highest dose (10%) having the greatest effect. The same effect was observed for food conversion efficiency calculated after 4 weeks.

(2) No changes were observed in the blood (red blood cells, white blood cells, hemoglobin, hematocrit).

(3) No abnormality was seen in the urine.

(4) Significant differences appeared in blood glucose levels (slight decrease) and blood urea nitrogen (slight increase) between the control and test groups given higher doses of the HPP. Alkaline phosphatase values were also slightly elevated at the highest dose level.

(5) Liver, kidney, and testicle weights were higher in groups fed the largest dose of HPP than in controls.

Table 88 (0249)

Maggi HPP 4BE: 90-day Trial with Rats

Groups

A. Basal casein diet/20% protein
B. Basal casein diet/20% protein + 1% Maggi powder 4BE
C. Basal casein diet/20% protein + 2.5% Maggi powder 4BE
D. Basal casein diet/20% protein + 5% Maggi powder 4BE
E. Basal casein diet/20% protein + 10% Maggi powder 4BE
G. Basal casein diet/20% protein + NaCl, in amounts equal to 5% diet D
H. Basal casein diet/20% protein + NaCl, in amounts equal to 10% diet E
J. Basal casein diet/20% protein + NaCl, NH_4Cl and MSG in amounts equal to 5% diet D
K. Basal casein diet/20% protein + NaCl, NH_4Cl and MSG in amounts equal to 10% diet E

Groups	A	B	C	D	E	G	H	I	K
Casein	23.85	23.45	22.92	22.05	20.30	23.85	23.85	23.85	23.85
Salt	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamins	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Oil	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Sugar	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Maggi	---	1.00	2.50	5.00	10.00	---	---	---	---
Cornstarch	46.15	45.55	44.58	42.95	39.70	44.17	42.19	43.43	40.70
Potato starch	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
NaCl	---	---	---	---	---	1.98	3.96	1.98	3.96
NH_4Cl	---	---	---	---	---	---	---	0.06	0.13
MSG	---	---	---	---	---	---	---	0.68	1.36

All diets contain 20% protein; appropriate adjustments were made in the amounts of casein in diets B, C, D and E, to take into account the protein contributed by Maggi powder 4BE.

(6) In the group receiving the highest dose (10% HPP 4BE) there were only rare abnormalities observed in the liver. The kidneys in most males and half of the females showed dilation of renal tubules with or without subsequent focal epithelial hypertrophy. There were no other serious renal changes observed.

The study concluded that no clinical symptoms of significance developed in rats ingesting the HPP at these lower levels for 90 days and that this particular hydrolyzed protein (Maggi HPP 4BE) had no deleterious effects when fed at these levels.

C. Humans

1. Levey et al. (4364 see also page 194) studied the toxic effects on human subjects following i.v. administration of three protein hydrolysates (an acid hydrolysate of casein with added tryptophan; an enzymatic hydrolysate of casein; and an enzymatic digest of beef blood proteins) compared with similar solutions containing partly neutralized glutamic acid.

They found that fewer subjects receiving the latter solutions developed nausea and vomiting at elevated glutamic acid blood levels than among those receiving the protein hydrolysates. (The group given protein hydrolysates also received two amino acid mixtures.) The authors interpreted these results as indicating that the presence of other amino acids might have a potentiating effect on the toxicity of glutamic acid.

2. As the result of further study with humans to determine which amino acids were responsible for producing nausea and vomiting when administered i.v., Smyth et al. (6878) concluded that decreased tolerance to casein hydrolysates was probably due to the presence of free glutamic and aspartic acids. These two amino acids constituted about one-third of the amino acid

content of the casein.

3. Another effect of casein hydrolysate studied by Smyth and coworkers (6879) was on the appetite of normal men. They observed that enzymatically hydrolyzed casein had little effect after either oral or i.v. administration but acid-hydrolyzed casein consistently produced a marked depression in voluntary food consumption after i.v. administration. A mixture of the 10 essential amino acids plus glycine had no appetite depressing effect.

4. Coppinger et al. (1500) reported a case of fatal allergic shock 60 minutes after a man (white, age 44) received 4 ml of a casein hydrolysate (Amigen) parenterally. Since passive transfer tests with the patient's serum were positive and the particular solution of hydrolysate administered was not found to be allergenic when tested with guinea pigs and normal persons, it was assumed that the death was an atopic reaction.

The authors suggested, therefore, that before individuals were given protein hydrolysates parenterally, they should be tested for sensitivity to them.

III. Special Studies

Rats

1. Alston and Thomson (0186) investigated the changes in chemical composition and mitotic activity of the rat liver after i.v. or i.p. administration of a normal dietary amount of protein hydrolysate. Female albino rats (180 $\pm$ 20 g) were given an enzymatic casein hydrolysate (Aminosol Vitrum 10%) by either intravenous or

intraperitoneal infusion. Table 89 shows the effect on liver composition of protein hydrolysate administration over a 12-hour period. In the treated animals, the liver cells maintained their mass and increased their protein content and in particular, their RNA content, while in the liver cells of the control animals, there was little, if any, change in mass or protein content and RNA content was maintained. Tables 90 and 91 summarize the experimental results of the effect on liver composition and mitotic frequency after more prolonged treatment with the protein hydrolysate (44 and 48 hours, respectively). After both 44 and 48 hours, there was a pronounced increase in the RNA per cell in the test animals. The apparent change in the cell mass and protein content per cell of control and test animals in both experiments was not significant. A final result for both experiments was the production of a significant increase in mitotic activity.

2. Rosen and Milholland (6234) examined the effect of an enzymatic casein hydrolysate on the induction and regulation of tyrosine transaminase activity. Adrenalectomized and intact Sprague-Dawley rats were used for all experiments. The results of the various experiments are summarized in Tables 92-98, and Fig. 11.

(1) After ingestion of enzymatic casein hydrolysate by intact or adrenalectomized animals, a severalfold increase in the activity of tyrosine-α-ketoglutarate transaminase occurred, whereas tryptophan pyrrolase was unresponsive.

(2) No rise in tyrosine transaminase activity was produced by administration of tyrosine, tryptophan, or a combination of these two amino acids equivalent in amount to those contained in an effective dose of the casein hydrolysate.

Table 89 (0186)

Treatment	No. of animals		µg DNA-P/ g wet wt.	µg RNA-P/ µg DNA-P	µg protein/ µg DNA-P
Protein hydrolysate	6	Biopsy before treatment	254 ± 26	3.20 ± 0.17	594 ± 61
		Liver after treatment	247 ± 16	3.74 ± 0.19	640 ± 62
		% change	- 1.0 ± 4.2[a]	16.6 ± 1.2[b]	7.7 ± 2.9[c]
Glucose control	5	Biopsy before treatment	228 ± 10	3.43 ± 0.28	551 ± 50
		Liver after treatment	257 ± 21	3.51 ± 0.24	508 ± 37
		% change	12.8 ± 6.6	2.3 ± 1.5	- 7.7 ± 4.3

Effect of liver composition of 12 hours i.v. administration of protein hydrolysate. DNA-P, deoxyribonucleic acid phosphorus; RNA-P, ribonucleic acid phosphorus.
[a]Values are means for the group ± S.E.
[b]Change significantly different from zero ($P < 0.001$).
[c]Change significantly different from zero ($P < 0.05$).

Table 90 (0186)

Treatment	No. of animals		µg DNA-P/ g wet wt.	Mean[a] cell mass mg wet wt/µg DNA-P	µg RNA-P/ µg DNA-P	µg protein/ µg DNA-P	Increase[b] in mitoses per 100,000 nuclei
Protein hydrolysate	5	Biopsy before treatment	204 ± 13[c]	4.99 ± 0.69	2.73 ± 0.21	649 ± 36	
		Liver after treatment	171 ± 5	5.88 ± 0.35	4.08 ± 0.23	731 ± 82	
		% change	- 15.2 ± 5.3[d]	+19.6 ± 7.8	+51.6 ± 9.2[e]	+ 11.8 ± 8.2	442 ± 12[g]
Saline control	5	Biopsy before treatment	221 ± 11	4.58 ± 0.27	2.19 ± 0.10	485 ± 45	
		Liver after treatment	205 ± 7	4.88 ± 0.17	2.55 ± 0.05	487 ± 24	
		% change	- 5.4 ± 7.5	+ 8.4 ± 8.14	+17.0 ± 3.8[f]	+ 2.2 ± 5.6	60 ± 83

Effect on liver composition of 44 hours i.v. administration of protein hydrolysate. DNA-P, deoxyribonucleic acid phosphorus; RNA-P, ribonucleic acid phosphorus.
[a]Calculated on the assumption that the DNA content per cell remains constant.
[b]Calculated from the difference between biopsy taken before treatment and liver after treatment.
[c]Values are means for the group ± S.E.
[d]Change significantly different from zero $P < 0.05$.
[e]Change significantly different from zero $P < 0.01$.
[f]Change significantly different from zero $P < 0.02$.
[g]Change significantly different from zero $P < 0.001$.

Table 91 (0186)

Treatment	No. of animals		µg DNA-P/ g wet wt.	Mean[a] cell mean mg wet wt/µg DNA-P	µg RNA-P/ µg DNA-P	µg protein/ µg DNA-P	Increase[b] in mitoses per 100,000 nucle
Protein hydrolysate	4	Biopsy before treatment	250 ± 11[c]	4.02 ± 0.16	2.85 ± 0.06	405 ± 20	
		Liver after treatment	221 ± 14	4.59 ± 0.33	3.93 ± 0.03	476 ± 34	
		% change	- 11.8 ± 4.3	14.1 ± 7.0	38.5 ± 10.7[d]	18.3 ± 8.5	284 ± 84[d]
Saline control	4	Biopsy before treatment	251 ± 19	4.06 ± 0.31	2.99 ± 0.31	435 ± 70	
		Liver after treatment	252 ± 25	4.01 ± 0.20	3.16 ± 0.26	440 ± 32	
		% change	1.0 ± 3.4	- 0.75 ± 3.2	6.8 ± 3.9	2.0 ± 4.1	4 ± 6

Effect on liver composition of 48 hours i.v. administration of protein hydrolysate. DNA-P, deoxyribonucleic acid phosphorus; RNA-P, ribonucleic acid phosphorus.
[a]Calculated on the assumption that the DNA content per cell remains constant.
[b]Calculated from the difference between biopsy taken before treatment and liver after treatment.
[c]Values are means for the group ± S.E.
[d]Change significantly different from zero $P < 0.05$.

Table 92 (6234)

Comparison of Induced Responses of Several Enzymes in Livers of Adrenalectomized Rats Treated with Hydrocortisone Hemisuccinate and Casein Hydrolysate[a]

Compound and dosage per rat	Period between treatment and killing (hr)	Tyrosine transaminase (μmoles/mg protein/hr)	Serine dehydrase	Tryptophan pyrrolase (μmoles/g protein/hr)
None		0.68 ± 0.09	2.66 ± 0.32	10.6 ± 0.67
Hydrocortisone hemisuccinate, 5 mg	4	3.88 ± 0.22	3.35 ± 0.54	80.9 ± 2.9
Hydrocortisone hemisuccinate, 5 mg, + casein hydrolysate, 1 g	4	6.54 ± 0.24	4.65 ± 0.49	88.8 ± 8.2
Hydrocortisone hemisuccinate, 5 mg	8	2.04 ± 0.12	5.27 ± 0.34	56.5 ± 3.94
Hydrocortisone hemisuccinate, 5 mg, + casein hydrolysate, 1 g	8	3.59 ± 0.12	6.01 ± 0.28	69.1 ± 6.9

[a]The hydrocortisone hemisuccinate was given intraperitoneally and the casein hydrolysate orally as a suspension in 3 ml of NaCl solution. Results are expressed as the mean value ± S.E. for 5 rats weighing from 118-154 g.

Table 93 (6234)

Effects of Different Doses of Casein Hydrolysate on Tyrosine Transaminase and Tryptophan Pyrrolase Activities in Livers of Adrenalectomized Rats[a]

Treatment and dosage per rat	Tyrosine transaminase (μmoles/mg protein/hr)	Tryptophan pyrrolase (μmoles/g protein/hr)
Control	0.41 ± 0.04	11.9 ± 0.54
Casein hydrolysate, 0.5 g	1.03 ± 0.20	13.4 ± 1.4
Casein hydrolysate, 1.0 g	1.46 ± 0.18	12.5 ± 0.85
Casein hydrolysate, 2.0 g	2.05 ± 0.24	11.3 ± 1.0
Casein hydrolysate, 3.0 g	2.24 ± 0.15	13.1 ± 0.63

[a]An enzymatic digest of casein was given orally as a suspension in 3 ml of isotonic NaCl solution. Control animals received isotonic NaCl only. All animals were killed 4 hours after treatment. Results are expressed as the mean value ± S.E. for 5 rats weighing from 172-219 g.

Table 94 (6234)

Effects of Actinomycin D on Induction of Tyrosine Transaminase by Casein Hydrolysate in Livers of Adrenalectomized Rats[a]

Treatment and dosage	Period between start of treatment and killing (hr)	Tyrosine transaminase (μmoles/mg protein/hr)
Control	4	0.49 ± 0.06
Casein hydrolysate, 1 g per rat	4	1.62 ± 0.12
Actinomycin D, 1 mg per kg	4	0.29 ± 0.08
Casein hydrolysate, 1 g per rat, + actinomycin D, 1 mg per kg	4	0.52 ± 0.04
Casein hydrolysate, 1 g per rat, at 0 and at 4 hours	8	2.81 ± 0.24
Casein hydrolysate, 1 g per rat, at 0 and at 4 hours + actinomycin D, 1 mg per kg, at 4 hours	8	0.88 ± 0.12

[a]The casein hydrolysate was given orally as a suspension in 3 ml of NaCl solution; actinomycin D (1 mg) was injected intraperitoneally. Control animals were treated orally and intraperitoneally with equivalent volumes of NaCl solution. Results are expressed as the mean value ± S.E. for 4-6 rats weighing from 120-150 g.

Table 95 (6234)

Influence of Protein Intake on Induction of Tyrosine Transaminase and Tryptophan Pyrrolase by Cortisol and Casein Hydrolysate[a]

Treatment	0% protein diet		18% protein diet		95% protein diet	
	Tyrosine transaminase[b]	Tryptophan pyrrolase[c]	Tyrosine transaminase[b]	Tryptophan pyrrolase[c]	Tyrosine transaminase[b]	Tryptophan pyrrolase[c]
Control	0.46 ± 0.10	25 ± 1.5	0.37 ± 0.04	24 ± 1.6	2.4 ± 0.34	50 ± 2.7
Hydrocortisone, 2 mg per kg	0.74 ± 0.04	46 ± 5.2	0.87 ± 0.05	78 ± 4.1	3.7 ± 0.26	104 ± 6.1
Casein hydrolysate, 1 g per rat	1.6 ± 0.13	40 ± 1.3	1.6 ± 0.21	38 ± 1.8	2.8 ± 0.27	58 ± 4.4
Hydrocortisone, 2 mg per kg, + casein hydrolysate, 1 g per rat	6.2 ± 0.74	57 ± 1.5	3.8 ± 0.23	75 ± 4.1	3.9 ± 0.16	105 ± 12

[a]Intact rats, maintained on the diets for 7 days, were used. On day 7 the weights of the animals on the 0%, 18%, and 95% protein diets ranged from 87-113 g, 98-131 g, and 165-200 g, respectively. The treatments were given on the 7th day only. Cortisol was injected i.p. and casein hydrolysate was given orally; the animals were killed 4 hours after these treatments. Control animals were treated orally and i.p. with equivalent volumes of NaCl solution. Results are expressed as mean value ± S.E. for 4 or 5 animals per group.
[b]Values are expressed as micromoles of *p*-hydroxyphenylpyruvate per mg of protein per hour.
[c]Values are expressed as micromoles of kynurenine per g of protein per hour.

Table 96 (6234)

Comparative Effects of Casein Hydrolysate, Tryptophan, Tyrosine, or Tryptophan and Tyrosine on Tyrosine Transaminase and Tryptophan Pyrrolase Activities in Livers of Adrenalectomized Rats[a]

Treatment and dosage per rat	Tyrosine transaminase (μmoles/mg protein/hr)	Tryptophan pyrrolase (μmoles/g protein/hr)
Control, 3 ml of NaCl solution	0.61 ± 0.04	24.4 ± 2.7
Casein hydrolysate, 1 g	1.95 ± 0.17[b]	21.0 ± 1.6
L-Tryptophan, 14 mg	0.71 ± 0.02	22.9 ± 2.0
L-Tyrosine, 61 mg	0.70 ± 0.04	22.1 ± 2.8
L-Tryptophan, 14 mg, + L-tyrosine, 61 mg	0.80 ± 0.08[c]	24.0 ± 1.5

[a]Animals weighed 110-125 g. All compounds were given orally in 3 ml of isotonic NaCl solution. The animals were killed 4 hours after treatment. Results are expressed as the mean value ± S.E. for 5-7 rats in each group.
[b]$P < 0.01$.
[c]$P > 0.05$.

Table 97 (6234)

Comparison of Tryptophan and Tyrosine with Casein Hydrolysate on Response of Transaminase and Tryptophan Pyrrolase in Intact Rats[a]

Treatment and dosage per rat	Tyrosine transaminase (μmoles/mg protein/hr)	Tryptophan pyrrolase (μmoles/g protein/hr)
Control, 3 ml of NaCl solution	1.06 ± 0.04	29.1 ± 2.0
Hydrocortisone, 0.1 mg	1.24 ± 0.10[b]	52.3 ± 3.5
Casein hydrolysate, 1 g	2.74 ± 0.05	38.9 ± 3.7
Hydrocortisone, 0.1 mg, + casein hydrolysate, 1.0 g	3.53 ± 0.23	68.8 ± 2.9
Tryptophan, 14 mg, + tyrosine, 61 mg	1.72 ± 0.18[c]	43.5 ± 5.7
Hydrocortisone, 0.1 mg, + tryptophan, 14 mg, + tyrosine, 61 mg	2.18 ± 0.31	92.6 ± 3.4

[a]Animals weighed 110-125 g. Hydrocortisone was given i.p. and the casein hydrolysate and amino acids orally in 3 ml of NaCl solution. The animals were killed 4 hours after treatment. Results are expressed as the mean value ± S.E. for 5 animals in each group.
[b]$P > 0.05$.
[c]$P < 0.03$.

Table 98 (6234)

Selective Effects of Hydrocortisone and Casein Hydrolysate on Enzyme Induction[a]

Treatment and dosage	Tyrosine transaminase			Tryptophan pyrrolase			Radioactivity	
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 2	Exp. 3
	(μmoles/mg protein/hr)			(μmoles/g protein/hr)			(cpm/mg RNA)	
Control, 3 ml of NaCl solution	0.60 ± 0.03	0.69 ± 0.11	0.51 ± 0.04	17.9 ± 1.3	26.2 ± 4.7	19.0 ± 2.7	4754	2696 ± 149
Hydrocortisone acetate, 1 mg per kg	0.62 ± 0.09	0.93 ± 0.21	0.61 ± 0.05	34.0 ± 1.7	41.8 ± 3.5	30.1 ± 3.2	8807	3643 ± 135
Casein hydrolysate, 1 g per rat	1.59 ± 0.13	2.71 ± 0.33	1.16 ± 0.12	24.5 ± 1.6	33.7 ± 5.8	24.1 ± 0.85	5988	3703 ± 322
Casein hydrolysate, 1 g per rat, + hydrocortisone acetate, 1 mg per kg	2.57 ± 0.10	4.48 ± 0.25	2.62 ± 0.10	62.3 ± 5.5	66.6 ± 5.2	42.5 ± 4.2	7812	3869 ± 200

[a]Rats weighing from 125-175 g were used in these experiments. The control rats received 3 ml of NaCl solution orally. The casein hydrolysate was given by stomach tube as a suspension in 3 ml of NaCl solution, and the hydrocortisone i.p., at zero time. [6-^{14}C] Orotic acid was prepared in sterile NaCl solution and given i.p. 2 hours after the administration of the steroid and casein hydrolysate. The rats were killed 4 hours after the first treatment. In experiment 2, the rats were treated with 5 μC of the ^{14}C-labeled orotic acid and the RNA was isolated from a pooled sample of liver; in experiment 3, the dose of radioactive orotic acid was 3 μC and the RNA was isolated from individual livers. The values for both enzymes with the combination treatment were statistically significant ($P < 0.01$). For tyrosine transaminase, the differences obtained with cortisol were not significant ($P > 0.2$), but they were with casein hydrolysate ($P < 0.01$). The effect of cortisol on the activity of tryptophan pyrrolase, while of limited statistical significance ($P = 0.05$), is consistently observed; however, the differences seen following the administration of casein hydrolysate are quite variable and of a low order of significance ($P > 0.10$). On the basis of the incorporation of orotic acid into RNA (experiments 2 and 3), all treatments effected a significant increase ($P < 0.05$). Mean values ± S.E. for 4-5 rats in each group are given.

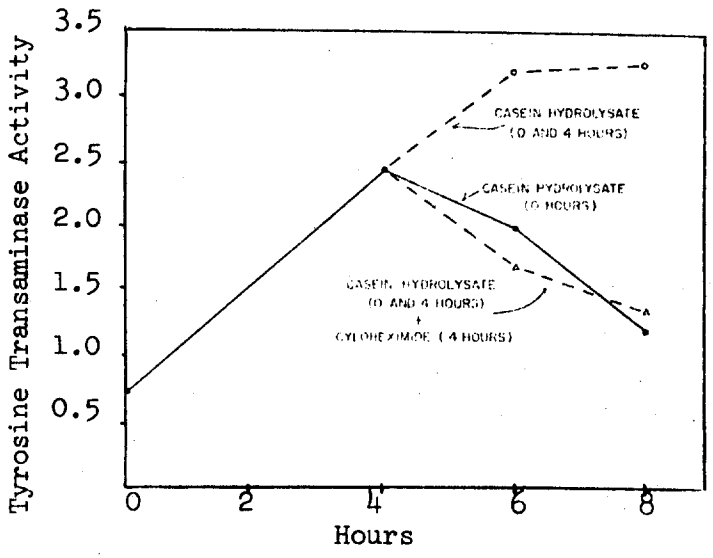

Fig. 11. Inhibitory effect of cycloheximide on the tyrosine transaminase response to casein hydrolysate in intact rats weighing from 99-119 g. All rats were intubated orally with 1 g of casein hydrolysate in 3 ml of isotonic NaCl at zero time. At 4 hours the rats were divided into three groups: ●—● , no additional treatment; o--o , a second intubation of 1 g of casein hydrolysate; △--△ , intraperitoneal injection of cycloheximide, 10 mg/kg, as well as a second intubation of 1 g of casein hydrolysate. Five rats from the control group (●—●) were killed at 4 hours and five rats from each of the three groups were killed at 6 and 8 hours. (6234)

(3) An indication that the synthesis of new RNA and protein was associated with the response of tyrosine transaminase to casein hydrolysate was inferred from the actinomycin D and cycloheximide experiments.

(4) When casein hydrolysate and hydrocortisone were given together or sequentially, additive and even synergistic effects were observed on tyrosine transaminase.

(5) When 1 g of casein hydrolysate was fed with a large dose of hydrocortisone, however, no effect on tryptophan pyrrolase or serine dehydrase occurred.

(6) A marked further increase in the activity of tyrosine transaminase was stimulated when adrenalectomized rats were given casein hydrolysate 4-8 hours after hydrocortisone treatment.

(7) When casein hydrolysate was fed together with a small dose of hydrocortisone to intact rats, tyrosine transaminase showed a synergistic response and tryptophan pyrrolase was also responsive. The authors attributed the latter effect, however, to tryptophan stabilization of the enzyme against degradation.

(8) A low dosage of hydrocortisone administered to intact rats produced an increase in tryptophan pyrrolase activity but not in tyrosine transaminase.

(9) Only tyrosine transaminase, of these two enzymes, responded to casein hydrolysate.

(10) Both casein hydrolysate and small doses of hydrocortisone stimulated the incorporation of [6-^{14}C]orotic acid into liver RNA to about the same extent. The authors interpreted this finding as a selective

effect, or that factors in addition to new RNA synthesis were involved in the induction of these enzymes (6234).

BIOCHEMICAL ASPECTS

I. Absorption

A. Rats

Peraino and Harper (5706) studied the concentrations of free amino acids in the portal and systemic blood plasma when rats were force-fed two proteins (zein and casein) and their hydrolysates. Male white rats (200 g, starved for 24 hours) were force-fed zein, casein, zein hydrolysate, or casein hydrolysate as aqueous suspensions or solutions (10% w/v) which added amounts of nitrogen equivalent to 1 g of pure protein (0.16 g N). At successive time intervals after feeding, blood samples were taken from the portal vein and heart, and the free amino acid content of the plasma quantitatively determined by paper chromatography.

It was found that much larger increases in plasma free amino acid concentrations occurred after feeding the protein hydrolysates than after the proteins. The largest increases were observed after ingestion of casein hydrolysate.

In their discussion the authors noted that the fact that the amino acids of zein and about one-half those of casein appeared more rapidly in the portal blood when the hydrolysates were fed, indicated that the rate of entry was limited by protein digestion more than by amino acid absorption.

However, some amino acids were not absorbed into the portal blood in direct proportion to their concentrations in the test diet. The authors explained this as follows:

(1) The rates of absorption of individual amino acids were found to be affected by the presence of other amino acids.

(2) The overall rate of absorption of an amino acid mixture as well as its pattern of absorption was affected by its composition at the site of absorption in the intestine. The presence of relatively large quantities of certain amino acids might depress the absorption of other amino acids.

(3) The rates of absorption of individual amino acids differed markedly, and this could influence the plasma amino acid pattern after feeding particular protein hydrolysates.

The authors concluded that the responses of the plasma amino acid pattern to the ingestion of protein hydrolysates were complex and depended on many other factors as well as digestibility of the dietary nitrogen source.

B. Humans

1. Stegink and Schmitt (7014) compared the serum amino acid levels of infants fed two types of protein-based formulas. Human infants (28-33 days old) were fed ad libitum, Nutramigen, a casein hydrolysate-based formula containing 22% free glutamate. Blood samples were drawn approximately two hours after feeding.

Postprandial serum amino acid levels are shown in Table 99. The authors commented that the decrease in glutamine levels in the Nutramigen-fed infants might be the result of the high intake of free glutamate, since other studies had reported decreases in plasma glutamine after ingestion of a glutamate load. Although there were significant differences in the concentrations of other amino acids (hydroxyproline, proline, alanine, valine, isoleucine, leucine, and lysine), serum glutamate levels were not elevated. The increased serum alanine levels in the Nutramigen-fed infants, however, were thought to reflect the glutamate content of the formula, because of reports that large

Table 99 (7014)

Postprandial Serum Amino Acid Levels in Young Infants Fed Cow's Milk Protein or Casein Hydrolysate-Based Formulas

Amino acid	Protein base: Cow's milk n = 24 (micromoles per 100 ml)[a]	Protein base: Casein hydrolysate n = 8 (micromoles per 100 ml)[a]	t	p
Taurine	11.4 ± 4.57	10.2 ± 3.07	0.67	0.5
Hydroxyproline	9.99 ± 2.81	5.36 ± 2.06	4.1	<0.002
Aspartate	1.39 ± 1.19	2.08 ± 1.49	1.3	0.2
Threonine	20.1 ± 3.99	23.4 ± 6.69	1.6	0.1
Serine + Asparagine	24.0 ± 4.71	27.6 ± 5.87	1.6	0.1
Glutamine	63.3 ± 7.61	56.2 ± 6.01	2.3	0.03
Glutamate	9.8 ± 2.80	10.1 ± 2.07	0.27	0.8
Proline	29.5 ± 6.84	39.8 ± 6.41	3.6	0.002
Citrulline	3.51 ± 1.07	3.63 ± 0.89	0.27	0.8
Glycine	34.2 ± 4.87	35.6 ± 8.14	0.59	0.6
Alanine	53.2 ± 12.7	69.9 ± 10.3	3.2	0.002
α-Aminobutyric	2.05 ± 0.42	2.16 ± 0.38	0.68	0.5
Valine	29.2 ± 5.99	40.2 ± 8.13	3.9	0.002
1/2 Cystine[b]	---	---	-	--
Methionine	4.36 ± 1.01	5.70 ± 1.28	2.9	0.01
Isoleucine	9.36 ± 1.70	13.3 ± 1.76	5.0	<0.002
Leucine	19.3 ± 3.38	24.1 ± 2.34	3.6	0.002
Tyrosine	11.5 ± 2.81	9.46 ± 1.96	1.8	0.07
Phenylalanine	10.3 ± 1.48	10.7 ± 1.56	0.56	0.6
Lysine	25.5 ± 5.11	35.4 ± 4.88	4.6	<0.002
Ornithine	18.5 ± 4.74	17.6 ± 3.34	0.50	0.6
Histidine	13.1 ± 1.67	14.0 ± 2.45	1.1	0.3
Arginine	13.6 ± 4.73	16.5 ± 5.43	1.4	0.2

[a]Expressed as mean ± 1 S.D.
[b]Cystine cannot be determined in serum samples.

amounts of ingested glutamate were partly converted to alanine during absorption. The authors concluded that the plasma amino acid levels after feeding were largely influenced by the nature of the ingested protein source.

2. Stegink and Baker (7013) studied the effect on plasma amino acid levels of two different protein hydrolysates (a casein hydrolysate, Amigen, and a beef fibrin hydrolysate, Aminosol) infused into six hospitalized infants. They reported that plasma glutamate and aspartate levels were within normal limits, but other amino acids were markedly below fasting levels. They noted that the amino acid composition of each protein hydrolysate preparation produced a characteristic plasma amino acid pattern.

The authors commented that a number of investigators had reported striking changes in plasma amino acid levels under conditions of dietary amino acid imbalance, and that subacute states of amino acid imbalance, toxicity, or antagonism might result from current formulations of protein hydrolysates. They felt, therefore, that the amino acid content of a protein infusion should resemble that of normal human plasma as closely as possible to achieve a better physiologic response in the patient.

II. Metabolism

Rats

1. The value of protein hydrolysates for treatment of protein deficiency was studied by Mital and Mathur (4989). They selected two enzymatic protein hydrolysates (soybean and groundnut hydrolyzed by papain) because such hydrolysates contained many peptides helpful in the synthesis of body tissues, whereas hydrolysates produced by acidic or alkaline hydrolysis contained only free amino acids.

Albino rats (180-200 g) were fed a basal protein-free diet or a diet containing 30% of dry soybean hydrolysate, dry groundnut (peanut) hydrolysate, or Proteinex (a proprietary protein nutritive preparation). Urine samples were collected at intervals and analyzed for total nitrogen.

Figure 12 summarizes the authors' conclusions that (1) turnover of tissue proteins, as indicated by urinary nitrogen, was higher with hydrolysates than with the protein-free diet, and (2) the soybean hydrolysate appeared to be the most rapidly absorbed, as evidenced by the output of urinary nitrogen with this hydrolysate. The soybean hydrolysate also showed superior growth-promoting values (see Table 100) and had the highest measured protein efficiency and net protein values.

2. In 1966 Ahrens and Wilson (8358) reported a 31-day study on 28-day specific-pathogen-free male rats fed a stock diet and then transferred to one of 4 chemically defined diets:

(1) High calorie with casein as the N source (N = 3% of diet).

(2) Same but with a mixture of L-amino acids simulating casein.

(3) Casein, with non-N ingredients reduced by one-third (restricted calorie diet).

(4) Restricted calorie diet, with amino acids.

Each rat received, weekly, i.p., 1 μC of glucose-1-C^{14}, -6-C^{14} or -U-C^{14} (0.036-0.06 mg). All label in urine, feces, and CO_2 was recovered.

Results and comments were as follows:

(1) By physical activity measurements (Table 101) casein-fed rats showed more voluntary activity than amino acid-fed rats ($P < 0.01$). High calorie rats had 70% of their activity at night, and restricted rats exercised evenly day and night.

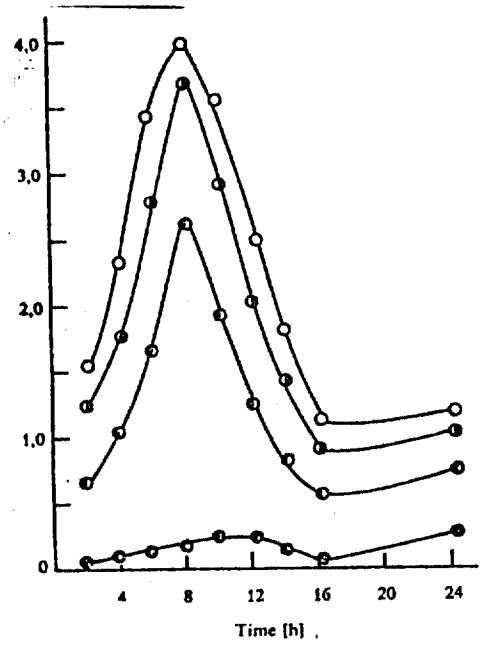

Fig. 12. Time relation of protein metabolism. ● Protein-free diet. ⊙ Protinex. ● Groundnut proteins. o Soybean proteins. (4989)

Table 100 (4989)

Growth-Promoting Values of Protein Diet on Rats

Protein sample	Gain in wt. (%)	Average protein efficiency	Net protein value
Soybean	40	2.08	19.1
Groundnut	35	1.82	15.4
Protinex	25	1.30	9.4

Table 101

Diurnal Variation in Revolutions Turned in Activity Cages Over a 3-Week Period by Rats Fed Nitrogen either as Amino Acids or as Casein, at 2 Levels of Calorie Intake (8358)

N source[1]	Intake[2]		Mean voluntary physical activity		
	N	Calories	12-hr light	12-hr dark	24-hr total
	g	kcal	rev	rev	rev
AA	8.59	1400	1379±456[3]	4143±701	5523±838
Casein	8.59	1425	1981±465	4230±736	6211±997
AA	8.93	982	4584±1023	3961±417	8544±1153
Casein	8.94	1040	6074±1169	4964±1050	11,038±1158

1 AA = amino acid mixture simulating casein.
2 Mean dietary intake/rat over the entire 31-day experimental period.
3 SE of mean; 6 rats/group.

(2) High calorie rats on casein gained more weight than those on amino acids ($P < 0.05$), and also more nitrogen per g of N intake ($P < 0.05$); both gains were reduced by calorie restriction ($P < 0.01$). Casein produced a non-significant increase of efficiency and storage of N compared with amino acids.

(3) Excretion data are shown in Tables 102 and 103 and Fig. 13. At 23 hours the nitrogen source affected the transfer of carbon 1 to CO_2 but not of carbon 6 or universally labeled carbon. Calorie level made no difference except for carbon 1, despite lower shunt activity with the calorie restricted diet.

(4) Casein diet was consumed more rapidly than amino acid diet. The data as a whole were interpreted as not consistent with the theory that voluntary activity reflected the state of energy metabolism.

(5) Less label was excreted in urine from carbon 1 than from carbon 6 or universal label; most urinary label (30%) was bound to citrate. Casein generated more urinary citrate label than did amino acids, and also more total specific activity in urine. Thus rats fed amino acids derived more of their energy from direct oxidation of glucose than did rats fed casein, as also was inferred from the CO_2 data. However, the amino acid rats could have diluted their citrate label by metabolism of unlabeled ($2NH_2$)-citrate, since their total citrate intake was double their urinary citrate output.

The authors also commented that the exact operation of the TCA cycle in these rats was uncertain, and there was a possibility that the glutamine, glutamate, arginine, and aspargine fed separately were less available than when fed in casein.

3. In 1973 Itoh *et al.* (8361) compared intact casein with an amino acid mixture at various feeding levels. When rats were fed continuously, intakes

Table 102

Percentage of Injected ^{14}C from Glucose Recovered in Expired $^{14}CO_2$, Feces, and Urine During the First 23 Hours Following Injection (8358)

N source [1]	Intake [2]		Glucose-1-^{14}C			Glucose-6-^{14}C			Glucose-U-^{14}C			
	N	Calories	$^{14}CO_2$	Feces	Urine	$^{14}CO_2$	Feces	Urine	$^{14}CO_2$	Feces	Urine	Urinary citrate [4]
	g	kcal	% injected ^{14}C			% injected ^{14}C			% injected ^{14}C			
AA	8.59	1400	67.7 ± 4.4 [3]	1.1 ± 0.3	1.9 ± 0.4	38.0 ± 4.0	2.9 ± 0.5	12.8 ± 4.1	53.7 ± 6.3	2.8 ± 0.7	14.6 ± 4.4	3.7 ± 1.2
Casein	8.59	1425	53.1 ± 4.8 [5] *	0.6 ± 0.2	1.9 ± 0.3	37.4 ± 7.4	3.4 ± 1.3	12.5 ± 2.7	52.8 ± 6.3	2.0 ± 0.4	14.3 ± 3.5	5.5 ± 2.4
AA	8.93	982	63.4 ± 3.9	0.9 ± 0.4	3.1 ± 0.4	46.7 ± 10.2	3.0 ± 1.1	16.6 ± 5.5	63.4 ± 8.4	2.1 ± 0.7	16.6 ± 4.9	2.6 ± 1.3
Casein	8.94	1040	56.6 ± 4.9	0.4 ± 0.1	2.0 ± 0.2	33.0 ± 3.5	2.6 ± 0.9	18.9 ± 3.9	46.2 ± 5.3	2.6 ± 0.7	19.3 ± 5.3	5.7 ± 2.1

[1] AA = amino acid mixture simulating casein.
[2] Mean dietary intake/rat over the entire 31-day experimental period.
[3] SE of mean; 6 rats/group.
[4] Specific activities (μCi/mEq) were: AA, 1400 kcal = 0.037 ± 0.012; casein, 1425 kcal = 0.057 ± 0.025; AA, 982 kcal = 0.024 ± 0.012; casein, 1040 kcal = 0.052 ± 0.019; none of these values proved to be significantly different from the others.
[5] Designated means significantly different ($P < 0.05$).

Table 103

Mean Squares of the Analyses of Variance for $^{14}CO_2$ Recovery and Urinary N (8358)

Source of variation	$^{14}CO_2$ recovered: Labeled carbohydrate administered			Urinary N excretion
	Glucose-U ^{14}C	Glucose-6 ^{14}C	Glucose-4-^{14}C	
	(%/23 hr)^2	(%/23 hr)^2	(%/6 hr)^2	(mg/24 hr)^2
Blocks	354.17	514.33	233.32	148.00	479
E = energy level	13.78	29.10	0.95	425.97*	16,970**
S = nitrogen source	485.55	308.24	688.33*	1,226.23**	1,883*
ES	397.97	256.04	89.12	10.28	170
Error	238.60	199.87	84.08	74.67	446

** Significant ($P < 0.01$).
* Significant ($P < 0.05$).

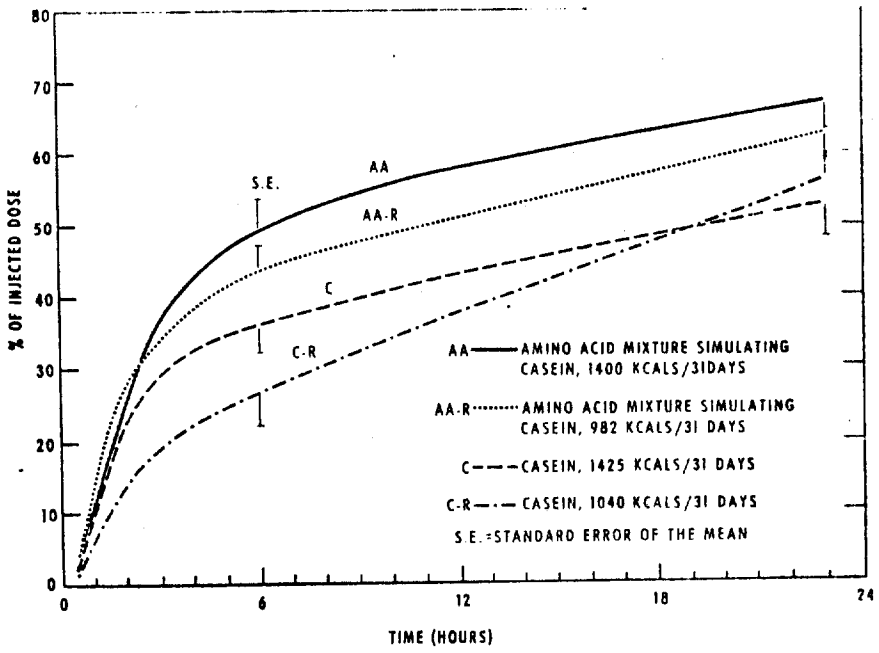

Fig. 13. Effect of calorie intake and nitrogen source on percentage of ^{14}C from intraperitoneally injected glucose-1-^{14}C recovered as $^{14}CO_2$. (8358)

and utilization of casein were again superior (8358 cited). But when rats were pair-fed for one hour twice daily, these differences vanished, and the mixture was found "nutritionally equivalent to the intact casein."

III. Consumer Exposure Information

The incorporation of protein hydrolysates into foodstuffs has the double purpose of improving the flavor and fortifying the nutritional content by adding a highly concentrated source of predigested protein. The flavor in general is described as meatlike (6063).

Two types of protein hydrolysates are largely used for food: (1) acid-hydrolyzed proteins; (2) and enzyme-hydrolyzed proteins. The protein source affects the resulting flavor, and the degree of flavor enhancement is apparently directly related to the glutamic acid content. Table 104 shows the approximate glutamic acid content of several sources of hydrolyzed protein. The protein sources used commercially are wheat gluten, corn gluten, extracted soya bean flour, casein, peanut flour, yeast, dried distiller's solubles, extracted cottonseed meal, fish waste, and sometimes egg albumin. Table 105 gives a typical analysis of a protein hydrolysate.

The foods in which protein hydrolysates are used vary from soups to fruitcake, as is shown in Table 106 (2752).

Hydrolyzed plant proteins (HPP) are also used to intensify, blend, or suppress other flavors in a wide variety of foods: canned, frozen, or dehydrated foods such as soups, sauces, vegetables, salad dressings, casseroles, meats, or snacks. HPP is also used to replace MSG. Usage levels may vary up to 1.5% as consumed depending on the type of product and whether it is used as a seasoning, enhancer, or potentiator. At present, no limitations

Table 104

Approximate Glutamic Acid Content of Proteins in percent[a]

Wheat gluten	36.0
Corn gluten	24.5
Zein	36.0
Peanut flour	19.5
Cottonseed flour	17.6
Soya bean flour	21.0
Casein	22.0
Rice	24.1
Egg albumin	16.0
Yeast	18.5

[a]From this table it can be seen that one of the most acceptable protein materials is wheat gluten. Wheat gluten contains from 80-90% protein, is fairly adequate with respect to its amino acid content, and is high in glutamic acid. Casein is also an excellent protein with good glutamic acid content. Soya bean flour and yeast are good proteins. Extracted edible cracklings are a fairly good protein, but not very high in glutamic acid content.

Table 105

Typical Analysis of a Good Protein Hydrolysate

Moisture	2.2%
Sodium chloride	39.8%
Total nitrogen	6.8%
Amino nitrogen	4.7%
pH (10% solution)	5.2

Calculated amino acid composition

Amino Acid	As Is Basis Percentage
Arginine	2.0
Histidine	1.0
Lysine	1.4
Tyrosine	1.7
Tryptophan	Trace
Phenylalanine	2.4
Cystine	0.5
Methionine	1.2
Threonine	1.3
Leucine	2.9
Isoleucine	1.7
Valine	1.8
Aspartic acid	4.1
Glutamic acid	12.0
Equivalent to Monosodium Glutamate	16.03

Table 106

Uses for Protein Hydrolysates in Food Products

Soups	Headcheese	Bread
Stews	Mincemeat	Macaroni with meat sauce
Broths	Sausage meat	Poultry stuffing and basting
Bouillons	Curing compounds	Chow mein
Bouillon cubes	Goulash	Processed meat
Fish	Biscuits & crackers	Hash
Gravies	Fruit cake	Meat sauces
Scrapple	Spice mixtures	Hors d'oeuvre pastes
Sandwich spreads	Chop suey sauce	Cheese spreads
Pickle relishes	Cheese rarebits	Mayonnaise
Baked beans	Chili sauce	Dog foods
Pancake flour	Salad dressings	

have been set by the FDA on the level of their use (5689).

Certain baby food manufacturers have replaced the MSG in some of their products with hydrolyzed proteins (see Table 107). Commercially prepared broths, soups, and bouillon cubes, which contain unspecified amounts of hydrolyzed protein and MSG (and thus more free glutamate, aspartate, and cysteine) (5485), tend to be fed in the home to infants (3440). The additive effects of these compounds have been discussed in preceding sections, and the authors imply risks to infants that have not been measured (5491,3436).

Hall (2752) in a symposium discussion on protein hydrolysates suggested that more specific standards should be established in evaluating protein hydrolysates for all foods. In this way the best possible products of constant and comparable quality would be produced with an acceptable minimum equivalent MSG content.

Table 107. Potential Daily Intakes of NAS Appendix A Substances (Groups I and II) per Food Category Based on Food Consumption by Eaters Only (Numbers rounded off).

Food Category	Substance Name	Age	Potential Daily Intake, mg Average	High A	High B	Very High
Other grains (R)	Protein, animal, hydrolyzed	0-5 mo.	1.3	2.8	2.7	5.5
		6-11 mo.	2.8	6.2	5.4	12.4
		12-23 mo.	3.0	6.0	6.0	12.0
		2-65+ yr.	5.1	10.0	10.2	20.1
Other grains (R)	Protein, vegetable, hydrolyzed	0-5 mo.	25.8	53.8	41.0	85.7
		6-11 mo.	52.3	120.0	83.3	191.0
		12-23 mo.	57.5	114.8	91.7	182.9
		2-65+ yr.	98.9	194.5	157.4	309.9
Baked goods (R)	Protein, vegetable, hydrolyzed	0-5 mo.	77.2	255.7	671.7	2223.0
		6-11 mo.	143.0	271.7	1243.6	2361.7
		12-23 mo.	273.7	447.5	2379.0	3891.3
		2-65+ yr.	684.3	1016.2	5949.8	8835.8
Fish products	Hydrolyzed vegetable protein	0-5 mo.	7.8	14.4	14.4	26.5
		6-11 mo.	10.0	18.8	18.4	34.5
		12-23 mo.	21.5	38.4	39.5	70.4
		2-65+ yr.	46.2	87.7	84.8	161.0
Processed vegetables	Protein vegetable, hydrolyzed	0-5 mo.	7.7	14.8	9.1	17.6
		6-11 mo.	28.4	55.2	33.8	65.7
		12-23 mo.	38.0	61.9	45.2	73.5
		2-65+ yr.	80.4	134.9	95.7	160.4
Soft candy	Vegetable protein, hydrolyzed	0-5 mo.	2.8	4.7	2.8	4.7
		6-11 mo.	3.8	8.5	3.8	8.5
		12-23 mo.	3.7	8.0	3.7	8.0
		2-65+ yr.	5.7	12.4	5.7	12.4

Table 107. (Cont'd)

Food Category	Substance Name	Age	Potential Daily Intake, mg Average	High A	High B	Very High
Fats, oils	Protein, vegetable, hydrolyzed	0-5 mo.	26.6	75.5	26.7	75.8
		6-11 mo.	22.3	45.1	22.3	45.2
		12-23 mo.	35.3	65.8	35.4	66.0
		2-65+ yr.	96.7	172.8	97.1	173.5
Milk products	Hydrolyzed vegetable protein	0-5 mo.	125.4	272.0	208.8	453.2
		6-11 mo.	315.3	959.6	525.2	1598.3
		12-23 mo.	202.7	738.5	337.6	1229.9
		2-65+ yr.	136.4	373.2	227.2	621.6
Cheese	Vegetable protein hydrolyzed	0-5 mo.	.9	1.7	1.9	3.2
		6-11 mo.	12.4	23.2	23.2	43.4
		12-23 mo.	16.4	35.4	30.6	66.2
		2-65+ yr.	18.5	38.6	34.6	72.1
Poultry	Protein, animal, hydrolyzed	0-5 mo.	9.4	23.4	10.5	26.3
		6-11 mo.	10.2	19.3	11.5	21.6
		12-23 mo.	12.0	20.4	13.5	22.7
		2-65+ yr.	19.1	36.8	21.4	41.1
Poultry	Vegetable protein, hydrolyzed	0-5 mo.	20.3	50.6	30.6	76.4
		6-11 mo.	22.0	41.7	33.3	63.0
		12-23 mo.	25.9	44.1	39.1	66.2
		2-65+ yr.	41.3	79.4	62.3	119.8
Egg foods	Vegetable protein, hydrolyzed	0-5 mo.	5.3	7.3	5.3	7.3
		6-11 mo.	9.7	16.3	9.7	16.3
		12-23 mo.	12.5	26.2	12.5	26.2
		2-65+ yr.	19.1	35.0	19.1	35.0

Table 107. (Cont'd)

Food Category	Substance Name	Age	Potential Daily Intake, mg Average	High A	High B	Very High
Soups	Animal protein, hydrolyzed	0-5 mo.	1.6	2.7	2.1	3.6
		6-11 mo.	18.6	43.0	24.9	57.7
		12-23 mo.	19.0	41.5	25.5	55.5
		2-65+ yr.	19.4	41.0	26.0	54.9
Gravies	Vegetable protein, hydrolyzed	0-5 mo.	9.4	17.7	16.8	32.0
		6-11 mo.	26.6	50.6	57.3	90.1
		12-23 mo.	60.1	120.0	107.0	213.9
		2-65+ yr.	114.0	226.2	203.2	403.4
Imitation Dairy	Vegetable protein, hydrolyzed	0-5 mo.	-	-	-	-
		6-11 mo.	84.7	106.6	166.0	209.0
		12-23 mo.	42.3	76.5	83.0	150.0
		2-65+ yr.	29.6	86.7	58.0	170.0
Formulas (baby)	Vegetable protein, hydrolyzed	0-5 mo.	8863.8	14361.6	10072.5	16320.0
		6-11 mo.	6426.2	13413.4	7302.5	15242.5
		12-23 mo.	7891.4	16361.4	8967.5	18592.5
Soups, Mixes (baby)	Vegetable protein, hydrolyzed	0-5 mo.	6.72	11.1	6.7	11.1
		6-11 mo.	15.5	36.7	15.5	36.7
		12-23 mo.	17.7	42.2	17.7	42.1
Meat Dinners (baby)	Vegetable protein, hydrolyzed	0-5 mo.	.6	.75	.6	.75
		6-11 mo.	3.9	9.7	3.9	9.7
		12-23 mo.	.9	1.8	.9	1.8
Combination dinner (baby)	Vegetable protein, hydrolyzed	0-5 mo.	57.6	114.0	134.4	266.0
		6-11 mo.	102.4	212.2	238.8	495.0
		12-23 mo.	97.7	216.1	227.9	504.3